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Petroleum Biodegradation Potential of Northern Puget Sound and Strait of Juan de Fuca Environments

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The oil-degrading activity of the microbial flora present in marine samples from three sites in the northern Puget Sound-Samish Bay, E. Fidalgo and Pt. Partridge and several sites in the Pt. Angeles area were investigated in this study. Activity was measured in terms of changes in the n-alkane and isoprenoid gas chromatographic profile of the satruate fraction and reported in terms of a "Degradative Capacity Index". Oil-degrading activity was greatest in areas adjacent to oil refineries and areas of relatively high levels of commercial activity. The levels of nitrogen and phosphorus were the primary environmental factors controlling the activity of the oil-degrading microbial flora. The fact that oil-degrading bacterial populations were readily isolated under enrichment conditions similar to those existing in the natural environment whereas fungi and yeast were only obtained under selective enrichment conditions (i.e. low pH) suggests that bacteria would be the most active group in removing oil spilled from this environment. Oil-degrading populations consisted predominantly of Flavobacterium and Pseudomonas genera with occasional populations containing a predominance of members of Acinetobacter and Alcaligenes genera.

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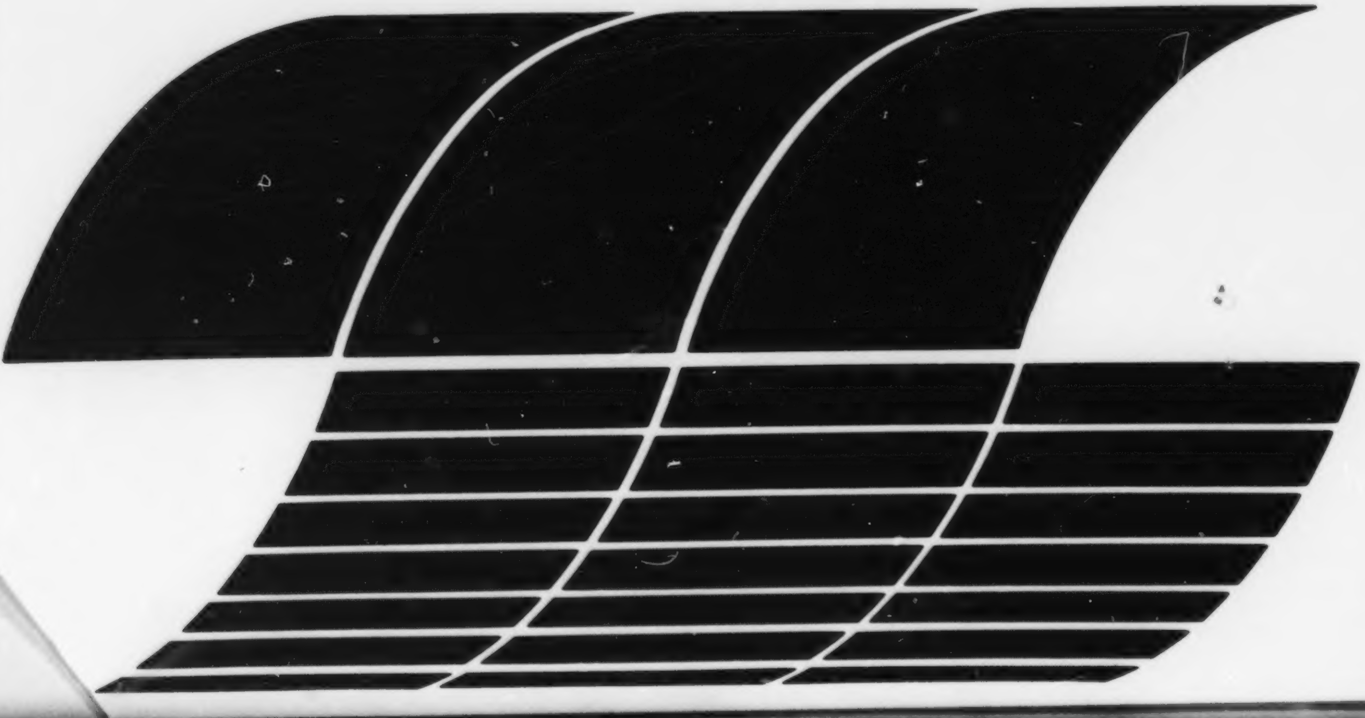

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by

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FOREWORD

Substantially increased petroleum tanker traffic, pipeline transport and refining operations are anticipated in the region of Northern Puget Sound and Strait of Juan de Fuca with the Alaskan pipeline in operation. To assess the potential future environmental impact arising from these activities, the Puget Sound Energy-Related Project contracted the University of Alberta to undertake a study on the "Microbial Degradation of Petroleum Hydrocarbons" in Northern Puget Sound and Strait of Juan de Fuca. This study was supported by U.S. Environmental Protection Agency "pass-through" funds administered by the NOAA Marine Ecosystem Analysis Program. This final report presents the results of two years of research.

ABSTRACT

The oil-degrading activity of the microbial flora present in marine samples from three sites in the northern Puget Sound - Samish Bay, E. Fidalgo and Pt. Partridge and several sites in the Pt. Angeles area were investigated in this study. Activity was measured in terms of changes in the n-alkane and isoprenoid gas chromatographic profile of the saturate fraction and reported in terms of a "Degradative Capacity Index". Oil-degrading activity was greatest in areas adjacent to oil refineries and areas of relatively high levels of commercial activity. The levels of nitrogen and phosphorus were the primary environmental factors controlling the activity of the oil-degrading microbial flora. The fact that oil-degrading bacterial populations were readily isolated under enrichment conditions similar to those existing in the natural environment whereas fungi and yeast were only obtained under selective enrichment conditions (i.e. low pH) suggests that bacteria would be the most active group in removing oil spilled from this environment. Oil-degrading populations consisted predominantly of _Flavobacterium_ and _Pseudomonas_ genera with occasional populations containing a predominance of members of _Acinetobacter_ and _Alcaligenes_ genera.

The highest rates of removal (i.e. weight loss) of Prudhoe Bay oil by the microbial flora present in water column materials were obtained in areas where maximum oil-degrading activity was observed (i.e. near refineries and commercially active sites). This loss in weight was due to the removal of compounds in the saturate and aromatic fractions of Prudhoe Bay oil. Components in the saturate fraction are slowly used (long lag phase) followed by an extended period of a high rate of removal whereas aromatics are continuously used at a low rate followed by a short period where a rapid loss of weight in this fraction occurs. Studies determining the rate of release of $^{14}CO_2$ from ^{14}C-labelled hydrocarbon-"spiked" Prudhoe Bay oil by the microbial flora present in water column and sub-tidal sediments showed that [9-^{14}C]-phenanthrene yielded the highest rates of $^{14}CO_2$ release followed by [1-^{14}C]-naphthalene, [1-C^{14}]-hexadecane and [9-^{14}C]-anthracene. However $^{14}CO_2$ was released very quickly (i.e. with a very short lag) from [1-^{14}C]-naphthalene-spiked oils indicating that the natural indigenous microbial flora very quickly adapted to this substrate. The shortest lag times for $^{14}CO_2$ evolution from all labelled substrates was observed with samples near oil refineries and commercially active areas. The highest yields of $^{14}CO_2$ (greater than 50%) were obtained with [9-^{14}C]-phenanthrene. Studies with glass capillary gas chromatographic analysis of the aromatic fraction indicate that substituted benzenes, naphthalene, and 1-methyl- and 2-methyl-naphthalenes were used before changes in the n-alkanes and isoprenoids were detected. It was also observed using this gas chromatographic technique that changes take place in the components of the aromatic and saturate fraction without nitrogen and phosphorus supplementation of marine samples although at a lower rate than when such nutrients were added.

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SECTION 1

INTRODUCTION

The Strait of Juan de Fuca and northern Puget Sound serve as the main transportation corridor for marine traffic proceeding to U.S. ports in the Puget Sound area (e.g. Seattle), and to Canadian ports (e.g. Vancouver). Until recently the incidence of oil-tanker traffic in this area was light, as the oil needs of the Pacific Northwest were being primarily supplied by pipeline from western Canada (1). However, as a result of the re-evaluation of Canada's oil needs, the Canadian government in the early 1970's re-allocated Canadian oil supplies so that western Canadian crude oil was no longer available to U.S. refineries in the Pacific Northwest area. The major refineries i.e. at Anacortes, Wash. (Shell Oil Co. and Texaco, Inc.) and at Cherry Point, Wash. (Mobil Oil Corp. and Atlantic Richfield Co.) now receive their crude oil exclusively via oil-tankers. A limited amount of oil is also delivered to refineries in the Vancouver area by oil-tankers. These developments have resulted in a tremendous increase in the volume of oil being transported by water in this area. When this is considered in conjunction with the general expansion of commercial marine traffic resulting from the increased uses of the Ports of Seattle and Vancouver together with the possibility of Pt. Angeles serving as a crude oil transhipment center there is an increased risk of an oil spill taking place.

Oil entering the marine environment can be removed from the water column by evaporation, or sedimentation either into inter-tidal or subtidal materials, or by biodegradation, or physical-mechanical clean-up. Only the components lost by evaporation (i.e. the low molecular weight volatile compounds) and physical-mechanical removal are not available for biodegradation. The rate of disappearance of oil from the marine environment depends on the physical and chemical properties of the oil spilled, and on chemical, mechanical, thermal and biological energies available in the system (2). It is the biological (i.e. microbiological) component of marine environmental systems on the southern shores of the Strait of Juan de Fuca and the northern Puget Sound area of the State of Washington which is the subject of this study.

Bacteria, yeasts and fungi (i.e. microorganisms) are unique biological species in that many of them have the genetic capability of using hydrocarbons either singly or in a mixture (as found in crude oil) as sources of food and energy. This capability has only occasionally been found in other forms of life (3). The microbial ability to degrade hydrocarbons is not a genetically stable characteristic; it is readily lost in Gram negative bacteria but is more stable in Gram positive bacteria, yeasts and fungi. If substrates (i.e. microbial food) like oil or hydrocarbons are continually introduced into a marine or terrestrial environment at low levels there will be a greater incidence of oil-utilizing microorganisms and a faster rate of removal of freshly added hydrocarbons than in areas where such additions are not taking place. The ubiquitous distribution of microorganisms in aquatic and terrestrial environments provides a biological system which contributes to the natural removal of oil spilled in the marine environment. In addition to the presence of microorganisms with oil-degrading capability, the oil spilled must be a biodegradable one (i.e. an oil which will sustain microbial growth) and environmental conditions have to be suitable for microbial growth. Parameters like temperature, pH, salinity, oxygen concentration, and nutritional factors such as nitrogen and phosphorus have to be in ranges which will support the growth of oil-degrading microorganisms. Any one of these parameters can control the rate of removal of oil by microbial activity. Information on the interaction of these factors affecting the activities of oil-degrading microorganisms in marine environments has been the subject of recent reviews (4, 5, 6) and a recent symposium (7). However, since no data are available on the Strait of Juan de Fuca and northern Puget sound areas of the Pacific Northwest, a study was designed to investigate factors affecting the activities and distribution of oil-degrading microorganisms under the environmental conditions prevalent in this area. The initial study (8) was designed to provide information on the effect of geographic and seasonal variation and proximity to oil sources on the activity, distribution and types of oil-degrading microbial flora found in waters, beaches and inter-tidal sediments of this area. The object of this study is to examine the microbiological oil-degradation process in greater detail in northern Puget Sound and Pt. Angeles area of the Strait of Juan de Fuca.

Investigations have been concerned with the enumeration of hydrocarbon utilizing microorganisms and the determination of their incidence as a fraction of the normal heterotrophic population. The problems encountered in such enumeration studies have been investigated by Walker and Colwell (9). Substrates used for enumeration techniques include pure hydrocarbons, synthetic oil mixtures (11), whole crude oils and whole crude oils spiked with ^{14}C-labelled hydrocarbons (10). Such experimentation has shown that oil- or hydrocarbon-polluted areas will have a greater incidence of hydrocarbon-utilizing microorganisms than adjacent unpolluted areas. Data obtained using pure hydrocarbons are difficult to evaluate regarding their value in assessing oil-degradation since it has been shown (11) that many fungi (e.g. Cladosporium resinae) which can grow on a pure hydrocarbon cannot grow on a whole oil containing that compound. Techniques based on the measurement of the increase in mass or number (e.g. plate count or Most Probable Number) of oil-degrading heterotrophs grown on a complex substance such as oil do not yield information regarding which compound or compounds are supporting growth. The use of ^{14}C-hydrocarbon-"spiked" oil yields the most valuable data but is limited by the availability of ^{14}C-labelled hydrocarbons. Growth also can be monitored indirectly by following changes in the concentrations of the compound(s) present (12). Of the four major components of crude oil i.e. asphaltenes, saturates, aromatics and the polar N (nitrogen)-, S (sulfur)-, O (oxygen)-containing molecules, only the n-alkanes (and to a lesser extent the isoprenoids) of the saturate fraction (13) and the mono-, di- and tri-ring compounds of the aromatic fractions (14, 15) are readily degraded by microorganisms. Changes in the content of n-alkanes and the isoprenoids can be readily monitored by gas chromatography (G.C.), while the resolution of aromatic compounds by G.C. requires more rigorous pre-treatment of the oil than is required for monitoring changes in the n-alkanes and isoprenoids. The approach used to assess distribution and the factors affecting the activity of oil-degrading microorganisms was based on an enrichment technique using crude oil (e.g. Prudhoe Bay) as sole carbon and energy source. Changes in the n-alkane and isoprenoid profile of recovered oil were determined by G.C. and used as an indicator of oil-degrading microbial activity. Such changes were used in the establishment of a "Degradative Capacity Index" for comparing the oil-degrading activities of microorganisms found in this marine environment.

The majority of techniques used for the enumeration of microorganisms employ conditions which favor bacterial growth but limit the development of yeast and fungi. Yet representative species of both these organisms are known hydrocarbon degraders and some grow on crude oil (11). The incidence and role of such organisms in the biodegradation process were investigated using selective enrichment and plating conditions followed by the examination of isolates regarding their oil-degrading capability.

The study of oil-degrading microorganisms has been shown to be influenced by temperature (16, 17) and by the nutrient status, especially the nitrogen and phosphorus content (18, 19, 20, 21) of the environment. The effects of both these parameters on the degradation of oil were studied in the initial investigation (8) and the effect of nutritional conditions was determined on all samples examined in this investigation.

The chemical composition of oil has been reported to have an influence on the biodegradation process (16, 22). In addition to oil from the north slope of Alaska, i.e. Prudhoe Bay, oil is received from many other areas (e.g. Malaysia and the Middle East) of the world (1). The possible effects of the differences in the physical-chemical composition of such oils on the biodegradation process were also investigated.

The rate of removal of oil from the marine environment will depend on the types of microorganisms which are present and the status of the physical-chemical parameters at the time the oil is introduced to the system. Some of the problems involved in the generation and the evaluation of oil-biodegradation rates were discussed in the early seventies (23). Two approaches, gravimetric and radiochemical (using Prudhoe Bay oil "spiked" with different ^{14}C-labelled hydrocarbons), were used in this study to obtain data on the rate of removal of oil and specific components of oil from water column material from pristine sites and sites which would be subject to hydrocarbon exposure. Data were also obtained with the radiochemical technique on the hydrocarbon-degrading activity of sub-tidal sediments and beach materials.

The initial studies (8) provided information on the distribution and factors affecting the activities of oil-degrading microorganisms in the marine environment of northern Puget Sound and the southern shore of the Strait of Juan de Fuca. This initial data base was expanded in these studies.

The oil-degrading capabilities of microorganisms in the water column, beach, inter-tidal and sub-tidal sediments from these areas were evaluated and the biodegradation process investigated. Particular emphasis was placed on obtaining data on the Pt. Angeles area, since there is a possibility of its development as a deep water oil tanker port. As our investigations were carried out in the laboratory the effect of oil on the microbial population under field conditions was studied in cooperation with the Battelle Institute at Sequim, Wash. A mini-study of the oil-degrading capability of water and sediment samples from the Duwamish River, which flows through the southern part of Seattle and empties into Elliot Bay, was also undertaken.

SECTION 2

CONCLUSIONS

Oil-degrading microorganisms are ubiquitous in the waters, beaches, inter-tidal and sub-tidal sediments of northern Puget Sound and the south shore of the Strait of Juan de Fuca. Their level of activity (as determined by loss of n-alkanes and isoprenoids) is quite variable, being greatest in areas adjacent to oil-refineries (e.g. Anacortes) or where relatively high levels of commercial and recreational marine activities take place (e.g. south shore of Pt. Angeles harbor). There was no relationship however between the total number of bacteria or yeast and fungi detected and the level of oil-degrading activity observed. The temperatures in this area are relatively constant and result in the presence of psychrotrophic (cold tolerant) and psychrophilic (cold-requiring) microbial flora. The levels of nitrogen and phosphorus are the primary environmental parameters which control the rate of activity of oil-degrading microorganisms in this marine system. Seasonal shifts in the observed levels of activity probably reflect in part natural nutrient cycling processes, e.g. phytoplankton blooms which could remove temporarily the available nitrogen and phosphorus. The oil-degrading microorganisms present in these environments consist of bacteria, fungi and to a lesser extent yeasts. Since oil-degrading bacterial populations (predominantly members of Flavobacterium and Pseudomonas genera with occasional populations containing a predominance of members of the Acinetobacter and Alcaligenes genera) are readily recovered under enrichment conditions similar to those which exist in the natural environment, it is projected that the bacteria would be the group most active in removing oil spilled in this environment. Oil-degrading fungi, and to a lesser extent yeasts, are also present and could function under conditions which retard or prevent the growth of bacteria.

Microbial activity (i.e. mineralization) can remove approximately 1/3 the weight of Prudhoe Bay oil; another 1/3 can be lost by weathering leaving a residue of 1/3 the original weight of oil. The highest rates of weight loss of Prudhoe Bay oil were obtained in areas where maximum oil-degrading activity was observed (i.e. near refineries and commercially active sites). Studies on the changes in the 4 major components of this oil (i.e. the asphaltenes, saturates, aromatics and polar N,S,O-containing compounds) indicate that loss in weignt was a result of utilization of aromatics and saturate components. The pattern of aromatic utilization suggests that components of this fraction are continuously used at a low rate with only short periods during which a rapid loss of weight of this fraction is observed. In contrast, components of the saturate fraction are initially slowly used (long lag phase) followed by an extended period when a high rate of weight loss is observed. There is a gradual increase in the proportion of polar N,S,O-containing compounds as a result of the microbiological degradation process.

This pattern of utilization of components of Prudhoe Bay oil was confirmed using carbon-14 labelled hydrocarbon-"spiked" oil and glass capillary gas chromatographic examination of recovered oil. The release of $^{14}CO_2$ from specifically labelled hydrocarbons added to Prudhoe Bay oil indicates that the maximum rate of removal of the hydrocarbons studied by microbes present in water and sub-tidal sediments was observed with labelled phenanthrene, followed by naphthalene, hexadecane and anthracene. This, coupled with the fact the $^{14}CO_2$ was released from [1-^{14}C]-nephthalene by microbes present in water column samples with a very short lag, supports the observation that certain aromatics are rapidly used, prior to the period of rapid n-alkane utilization, by the indigenous microbial flora. The shortest lag times were observed with samples taken near oil refineries and from commercially active harbor areas. The glass capillary gas chromatographic data indicate that the substituted benzenes, naphthalene, and 1-methyl- and 2-methyl-naphthalenes were being utilized prior to the catabolism of n-alkanes. Analysis of changes in the aromatic and n-alkane components with and without nutrient supplementation by this G.C. technique indicates that changes take place in these fractions without nutrient supplementation of marine samples, although at a lower rate than when exogenous nitrogen and phosphorus were present.

Pt. Angeles harbor area (Ediz Hook Pilot Station to Dungeness Spit) is a heterogeneous area regarding oil-degrading activity, with maximum activity observed in the harbor area (i.e. west of Morse Creek to Ediz Hook, Pilot Station). Water column samples from Peabody Creek were more active (i.e. shorter lag time) in releasing $^{14}CO_2$ from Prudhoe Bay oil "spiked" with specifically carbon-14 labelled hydrocarbons than water samples from Ediz Hook (Pilot Station) or Dungeness Spit. In contrast sub-tidal sediments from Ediz Hook (Pilot Station) were as active or more active than those from Peabody Creek. The activity of water column and sub-tidal samples from Dungeness Spit was generally lower than that observed with the other two sites studied.

The Duwamish River is an example of an aquatic system which has a very high level of oil-degrading activity present. Hydrocarbons were readily visible at some sites as a sheen on the surface of water as well as being extruded from sediments when samples were being taken.

The data presented in this and the previous report (8) are interpreted as showing the ubiquitous presence of oil-degrading microorganisms in the marine system investigated. Changes in an actual oil spill at the northern end of Vancouver Island are cited as revealing the potential of marine environments in this Pacific Northwest area to recover from oil pollution.

SECTION 3

RECOMMENDATIONS

One of the main difficulties in defining the oil-degrading capability of the marine environment is that it is a diverse, multiphasic, open system (i.e. consisting of water, inter-tidal and sub-tidal sediments and beach materials). Oil entering this environment may spread or partition in any or all of the components of this system. Each phase has different physical-chemical parameters like pH, dissolved oxygen level, temperature, nitrogen and phosphorus levels etc. which can influence the activity of oil-degrading microorganisms. As an open system these parameters are continuously changing, although at different rates for the phases under consideration, so observations made are relevant to the type of sample and the time and conditions existing when they are taken. In particular, the water column and inter-tidal sediments represent phases where the physical-chemical parameters are continuously changing whereas beach (i.e. at and above the high tide level) and sub-tidal materials represent more stable environments. The fate of oil incorporated into sub-tidal sediments is of particular interest as the environmental parameters existing there (e.g. low oxygen level) would result in the slow biodegradation of such oil. Under these conditions partially oxidized aromatics could be generated, resulting in a greater chance of generation of mutagenic compounds.

The main natural factors involved in the recovery of an oiled temperate marine environment are physical-mechanical ones (vigorous wave and tidal action) and microbial degradation. Physical-mechanical processes will be most operative in high energy areas like those found on the west side of Dungeness Spit and Pt. Partridge, but will have less effect in low energy areas like E. Fidalgo or depositional zones like Skagit Bay. It is in these latter stable, low energy areas that microbial degradation of oil, although a slow process under natural conditions, would assume more importance in removing oil spilled in the marine environment. If there is a possibility that such areas could be exposed to oil spilled from an existing or planned pipeline then the physical-chemical and biological parameters existing in such systems should be investigated. Such knowledge would be of use in accelerating the rate of recovery of such environments from the effects of an oil spill.

Information obtained to date on the marine environment as found in the northern Puget Sound and the south shore of the Strait of Juan de Fuca area indicate a ubiquitous distribution of oil-degrading microorganisms, although their activity varies markedly depending on the geographic location of the site being investigated. While a few low energy areas were studied in these investigations more information is required on the effect of microorganisms on oil in inter-tidal and sub-tidal sediments from such areas. Data on fuel oil spills in Buzzard's Bay, Mass. (42) indicate that aromatic compounds can persist in sediments for relatively long periods of time. In particular, the possible production of mutagens and the production and fate of aromatics and compounds which are recovered as N,S,O's should be investigated. The fate of asphaltenes, the most recalcitrant fraction of oil, should also be monitored, if a meaningful assay for determining changes in this fraction can be developed. Investigations should include studies on the effect of varying parameters (e.g. oxygen level and nutrient level-nitrogen and phosphorus) on the rate of removal of oil from such inter- and sub-tidal environments. Studies should be carried out under conditions as closely related to those found in the natural environment as possible (e.g. as per the Battelle-Sequim experiment) and under laboratory conditions. The latter studies are particularly important as the effect of physical-chemical parameters can be more closely monitored under laboratory conditions than under the "in vivo" conditions of the Battelle-Sequim experiments. Such investigations should follow the qualitative and quantitative changes taking place in the oil and microbial population present. The oil recovered from both field and laboratory studies should also be monitored for the production of mutagens.

Since the chemical composition of oil can have a marked influence on the effect of oil on an environment, studies on the fate and effect of other oils being brought into this area should be investigated. In particular, as the present studies show that changes can be brought about in Prudhoe Bay oil by the microflora in marine samples without nutrient supplementation, long term studies should be carried out on the effect and fate of different oils in the various environments found in this area, e.g. Pt. Partridge (pristine area), Pt. Angeles harbor (a commercially contaminated area), Dungeness Spit (a high energy area) and Skagit Bay (a low energy area).

The information resulting from the studies outlined would provide data complementary to those already obtained and would improve the ability to predict how an oil spill would affect and be affected by the microbial flora present in this marine environment.

SECTION 4

MATERIALS AND METHODS

Sample Sites and Sampling Procedures

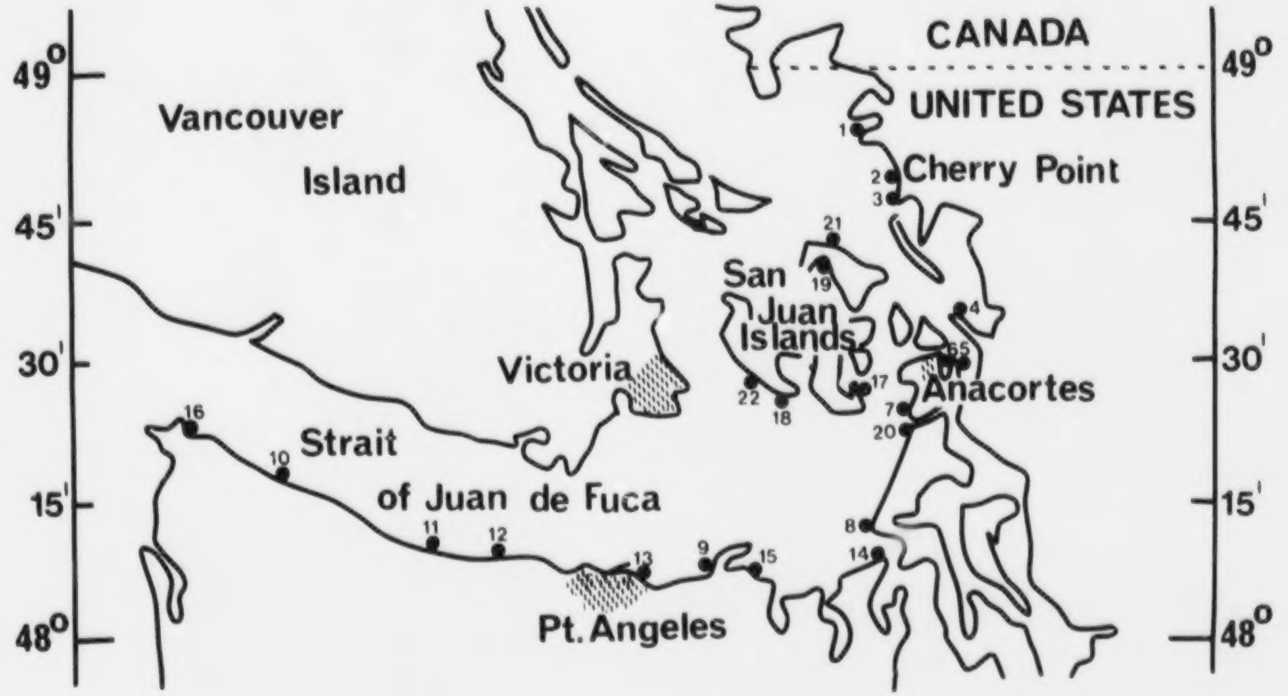
The locations of the sample sites used in the initial study (8) are shown in Figure 1 and those used in this investigation in Figure 2. The latitudes and longitudes of these sites are presented in Table 1. The initial sample sites were chosen to represent the diverse environments found in the northern Puget Sound and Strait of Juan de Fuca areas of the State of Washington and on the basis of their accessibility. The sites used in this study from the northern Puget Sound area were selected on the basis of the initial results (8) and represent the range of environmental types found in this area. The sites selected in the industrialized Pt. Angeles harbor are contrasted with sites chosen in the adjoining more "pristine" environments found in the Dungeness and Freshwater Bay areas. The environmental codes characterizing each site are available in NOAA's computerized data bank. Whenever tide and terrain permitted both a water column and a beach sample were obtained at each site.

Sub-tidal sediment samples were collected by a scuba diver. These were taken from Freshwater Bay and Dungeness Spit at a depth of approximately 6 m and from Peabody Creek and Ediz Hook Pilot Station at a depth of 13 m. Cores of inter-tidal sediments were obtained with sterile plastic (PVC) tubing (4.5 cm dia. x 35 cm) with one end beveled to facilitate coring. Tubes were carefully pushed vertically into sediment, capped, withdrawn, and transported to the laboratory. Such cores were sectioned into samples (10g) and assayed for oil-degrading activity and bacterial numbers.

Water samples were obtained at approximately 30 to 45 cm depth using a wide mouth 4L plastic bottle. Microbial and physical examinations were carried out on a sample which was obtained after rinsing the bottle 3 times with the water to be examined. Large volumes of water for laboratory use were taken and transported in sterile 4L bottles.

In this survey beach samples refer to sand obtained above the detritis (upper inter-tidal) left by spring tidal action. Such samples were a composite of 200 to 500 g of surface beach materials from approximately a 1 cm depth and 0.5 square meter area and were transferred using sterile tongue depressors to sterile 250 mL centrifuge bottles. Beach, inter-tidal cores and sub-tidal sediment samples were stored at -20°C unless otherwise indicated.

All materials were transported in cooler chests containing sufficient ice packs to keep the temperature at 10°C ± 5°C. Samples were obtained from sites in August, October and November 1978 and January, April, June and August 1979.


CANADA
UNITED STATES
Vancouver
Island
Cherry Point
San
Juan
Islands
Victoria
Anacortes
Strait
of Juan de Fuca
Pt. Angeles
49°
45'
30'
15'
48°
49°
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15'
48°
1
2
3
4
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Figure 1. Map showing location of sample sites used in 1977 - 1978 study.

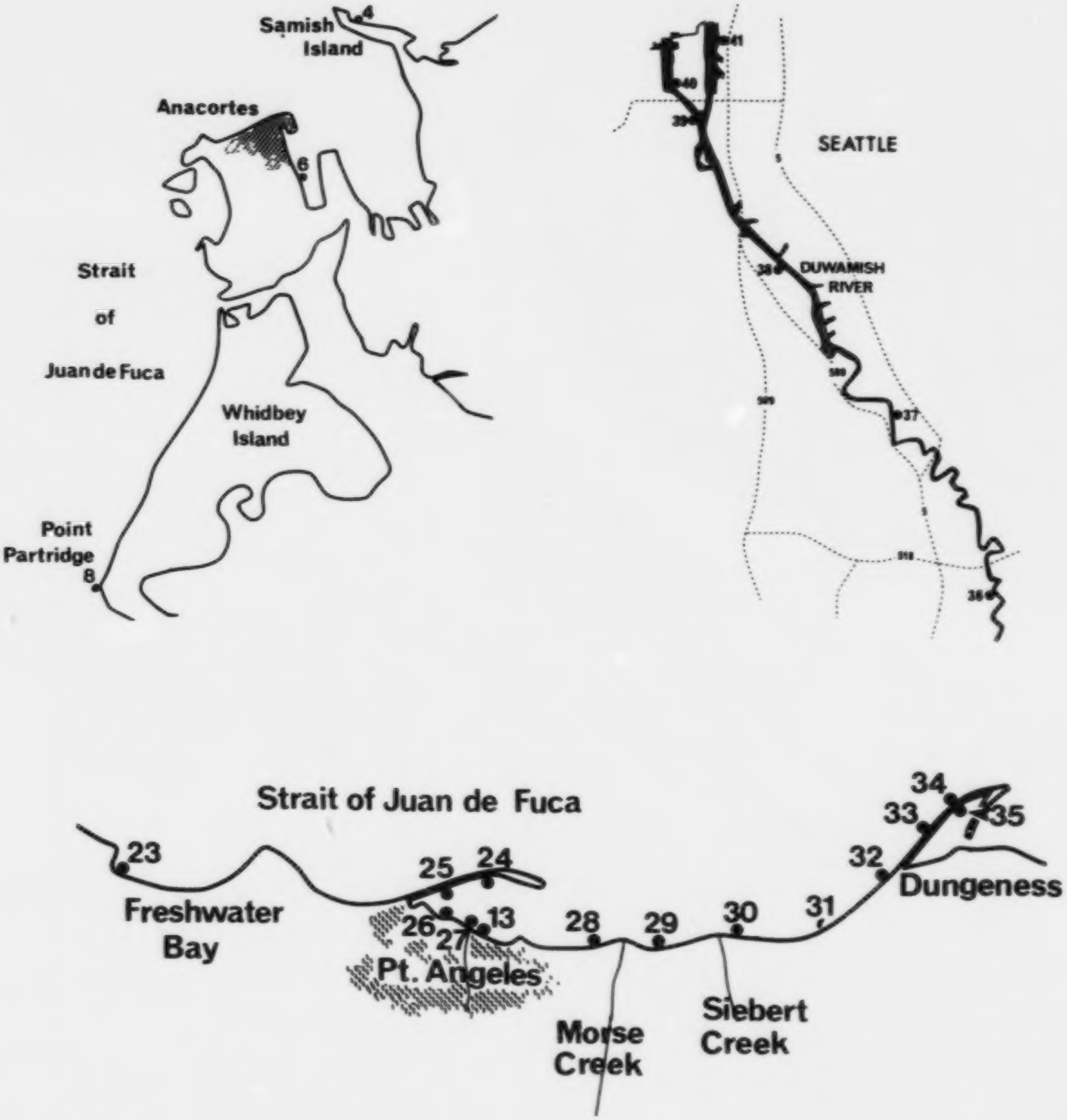
Samish Island
4
Anacortes
6
Strait of Juan de Fuca
Whidbey Island
Point Partridge
8
41
40
39
SEATTLE
5
38
DUWAMISH RIVER
599
509
37
5
518
36
Strait of Juan de Fuca
23
Freshwater Bay
25
24
26
27
13
Pt. Angeles
28
29
30
31
Morse Creek
Siebert Creek
32
33
34
35
Dungeness

Figure 2. Maps showing location of sample sites used in 1978 - 1979 study.

TABLE 1. SAMPLING LOCATIONS

Site No.[a]	Description	Latitude (N)	Longitude (W)
1	Birch Bay State Park	485405	1224610
2	Between Atlantic Richfield and Mobil Docks (refinery site)	485108	1224500
3	Beach Access of Sucia Road	485124	1224349
4	Samish Island Public Beach	483450	1223235
5	2 miles east Jct. 20W and East March Point Road	482930	1223320
6	Mud Flats 2 miles from Jct. East March Point Road and East Fidalgo Road	482930	1223550
7	Rosario Beach	482510	1223935
8	Point Partridge Park	481330	1224605
9	Entrance Dungeness State Park	480935	1230900
10	Clallam Bay	481540	1241720
11	Pillar Point	481251	1240603
12	Salt Creek Recreation Campground	481000	1234217
13	Port Angeles Southside #3 (Below Red Lion Inn)	480710	1232525
14	Fort Worden State Park	480845	1224525
15	Jamestown	480831	1230623
16	Neah Bay	482337	1243821
17	Lopez Island - Shoal Bight	482737	1224906
18	San Juan Island - South Beach	482723	1230013
19	Orcas Island - Crescent Beach	484141	1225351
20	Deception Pass State Park-West Beach	482408	1223946
21	Orcas Island - Terrill Beach	484244	1225252
22	San Juan Island - False Bay	482915	1230352
23	Freshwater Bay (Western Edge)	480829	1233755
24	Pt. Angeles-Ediz Hook #1 (Pilot Station)	480829	1232529

Table 1. continued ....

Site No.[a]	Description	Latitude (N)	Longitude (W)
25	Pt. Angeles-Ediz Hook #2 (Between Piles)	480823	1232653
26	Pt. Angeles Southside #1 (Public Marina)	480742	1232718
27	Pt. Angeles Southside #2 (Peabody Creek)	480714	1232542
28	West of Morse Creek	480650	1232232
29	West of Green Point (Near Cavern)	480650	1231917
30	Green Point #1 (East of Siebert Creek)	480702	1231634
31	Green Point #2 (West of Crevice)	480709	1231404
32	Green Point #3 (West of Dungeness Spit)	480811	1231207
33	Dungeness Spit #1 (Opposite First Navigational Marker)	480926	1231026
34	Dungeness Spit #2 (Opposite Second Navigational Marker)	481037	1230834
35	Dungeness Lagoon (Opposite Second Navigational Marker)	481037	1230834
36	Christiansen Rd. and Strandner Blvd. (Christiansen Green Belt Pk., Tukwila)	472715	1221430
37	119 St. S. East Side of Foot Bridge over Duwamish River.	472950	1221652
38	Monroe St. and 10th Ave. (Duwamish Waterway Pk.)	473153	1221903
39	S.W. Dakota and Duwamish River (Westside of Marina)	473402	1222057
40	Klikitat Ave. Opposite Fisher Mills (Longshoremans Parking Lot)	473424	1222116
41	Pacific N.W. Bell Cable Crossing (Southside)	473510	1222029

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[a] sites 1 to 22 used in 1977/1978 study; sites 4, 6, 8 and 13 and 23-41 inclusive were used in the 1978/1979 study.

Protocol for Measuring Oil-Degrading Activity

Water Column, Beach and Sediment Samples

The chemical compositions of all microbiological media used are rereported in Appendix A. An enrichment technique using a mineral medium with a neutral pH and Prudhoe Bay oil as sole carbon source was used to screen samples for the presence of oil-degrading microorganisms. The effect of nutrition (i.e. nitrogen and phosphorus levels) on oil-degrading activities of such populations was investigated using this technique. A similar procedure was used in assessing the biodegradability of Minas, Murban and Seria oils.

The oil-degrading activity of water column material was determined by setting up "in situ" duplicate enrichments consisting of 200 mL of water and 0.2 mL of Prudhoe Bay, Murban, Seria or 0.15 g of Minas oils in 500 mL screw-capped Erlenmeyer flasks. One of the duplicate flasks received 2.0 mL of a nitrogen and phosphorus supplement. To permit aeration the screw-caps were replaced in the laboratory with sterile foam plugs prior to incubation on a rotary shaker. In a laminar flow hood 10 g beach, inter-tidal cores or sub-tidal sediment material were removed aseptically from the field samples and added to 500 mL Erlenmeyer flasks each containing 200 mL of sterile artificial sea water medium (Appendix A) and oil. Duplicate flasks were prepared for each sample, one of which received a nutrient supplement of nitrogen and phosphorus, and all flasks were incubated on a rotary shaker. Unless otherwise specified all rotary shakers were run at 250 r.p.m. with an eccentricity of 3.8 cm and at a temperature of 8°C. After a suitable incubation period the oil was recovered by pentane flotation technique and the pentane-soluble fraction subjected to gas chromatographic (G.C.) analysis.

Oil-degrading activity was determined by comparing the G.C. profile of the saturate fraction of recovered oils with that of undegraded Prudhoe Bay oil (i.e. sterile controls) subjected to the same incubation and recovery procedures. Typical G.C. profiles for Prudhoe Bay oil at various stages of degradation are shown in Figure 3. To aid in evaluating bacterial oil-degrading activity each profile was given a numerical value as follows: a completely degraded n-saturate fraction (i.e. C) equal to 4; presence of residual isoprenoids (i.e. I) equal to 3; partial reduction of n-alkanes profile (i.e. P) equal to 2; selective removal of n-alkanes, C_{12} to C_{19} in carbon content (i.e. S) equal to 1; no degradation of n-alkanes (i.e. N) equal to 0. Thus, for each sample type from each sample site, a "Degradative Capacity Index" was calculated by summing the numerical values and dividing by the number of samples. Not enough data are available to set up a similar system for Minas, Murban and Seria oils. Utilization patterns with these oils can only be subjective and are reported on a plus or minus basis for utilization (Figures 4, 5 and 6).

Fungi and Yeast

The oil-degrading capability of pure yeast and fungal cultures was initially tested by the technique used by Davies and Westlake (11) with the

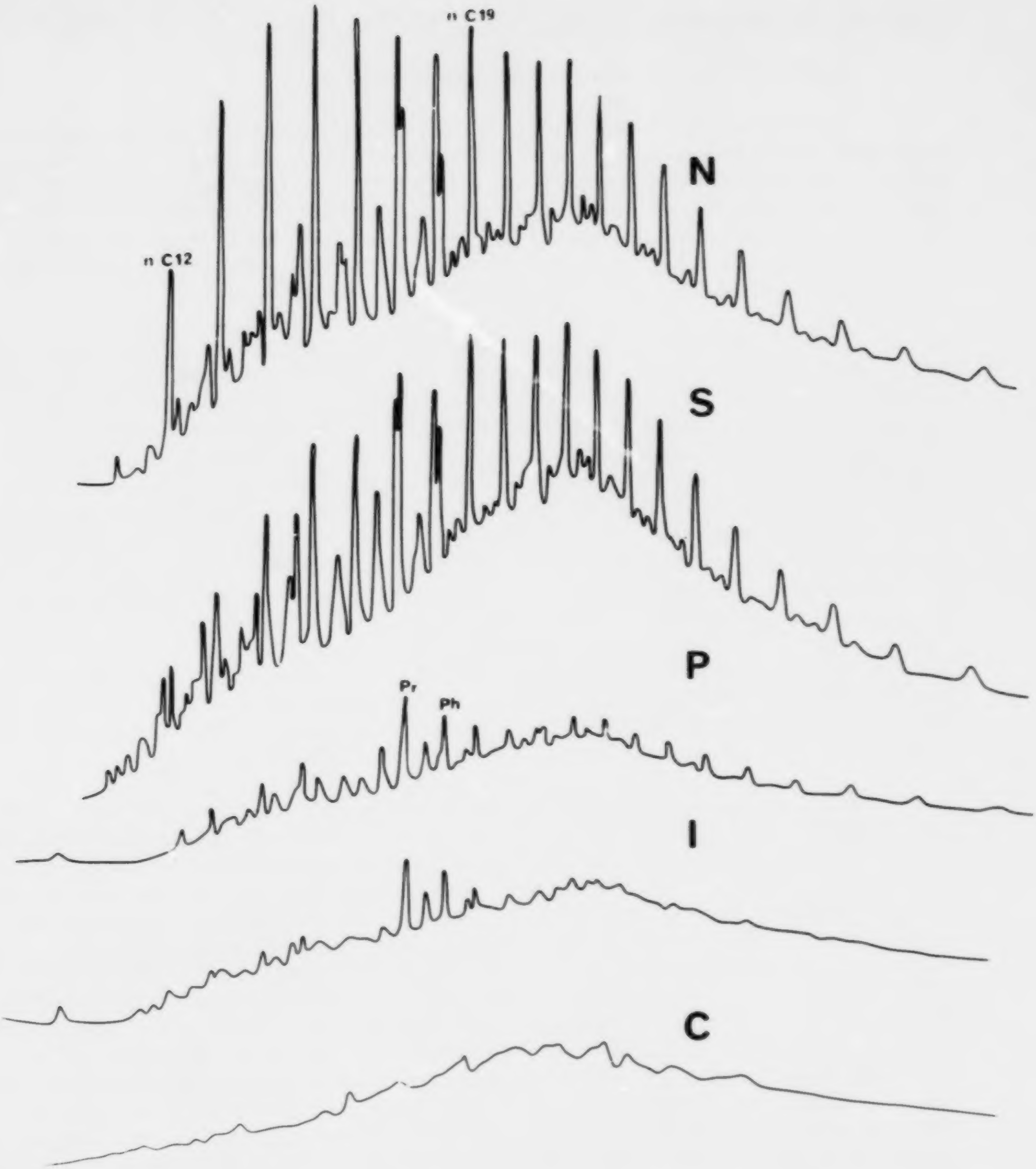

Figure 3. Typical G.C. profiles of saturate fraction of Prudhoe Bay oil before and after growth of enrichment populations (N = no degradation; S = selective metabolism, n-alkanes C_{12} to C_{19}; P = partial removal, n-alkane peaks still discernible; I = only isoprenoids remaining; C = complete utilization of n-alkanes and isoprenoids).

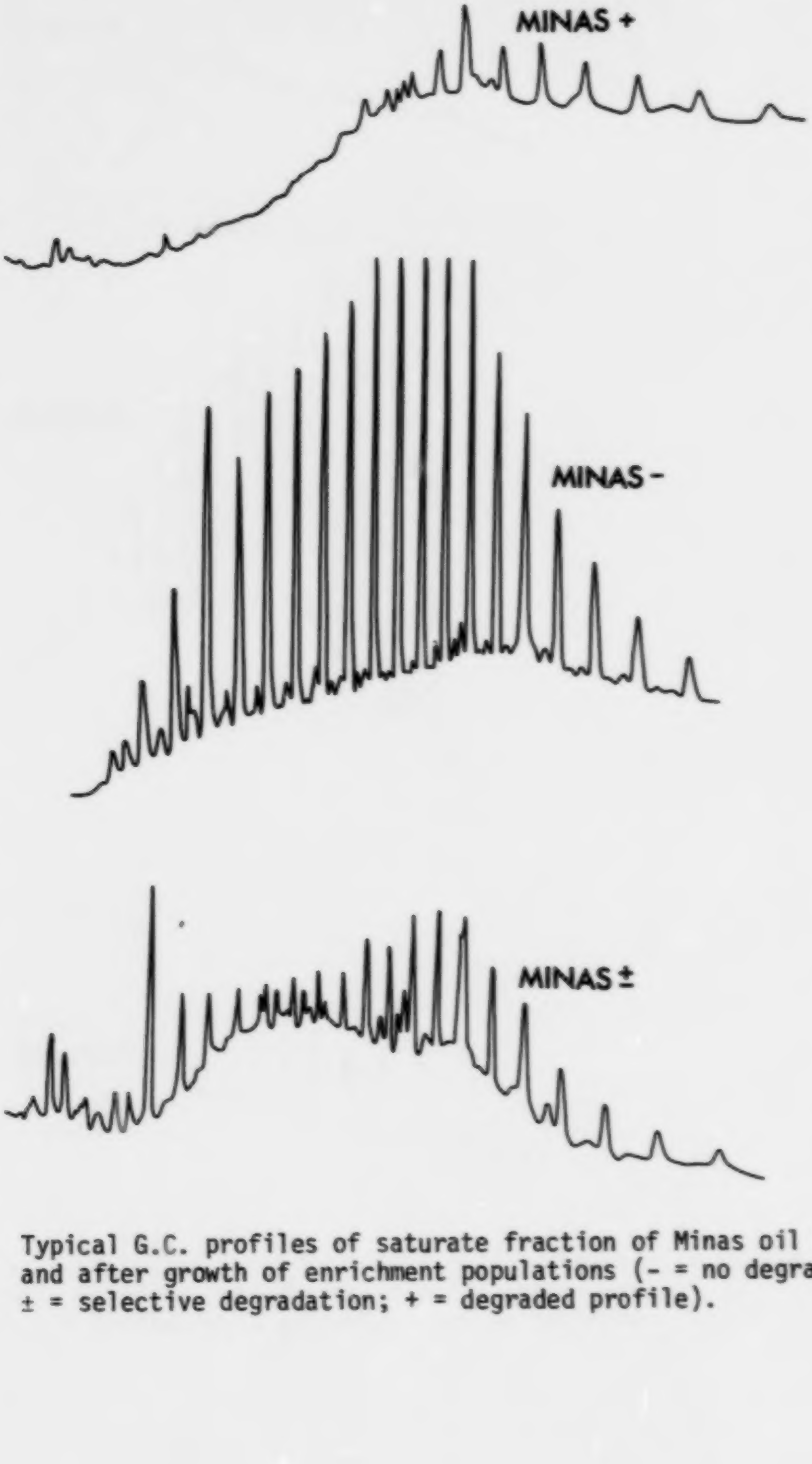

Figure 4. Typical G.C. profiles of saturate fraction of Minas oil before and after growth of enrichment populations (- = no degradation; ± = selective degradation; + = degraded profile).

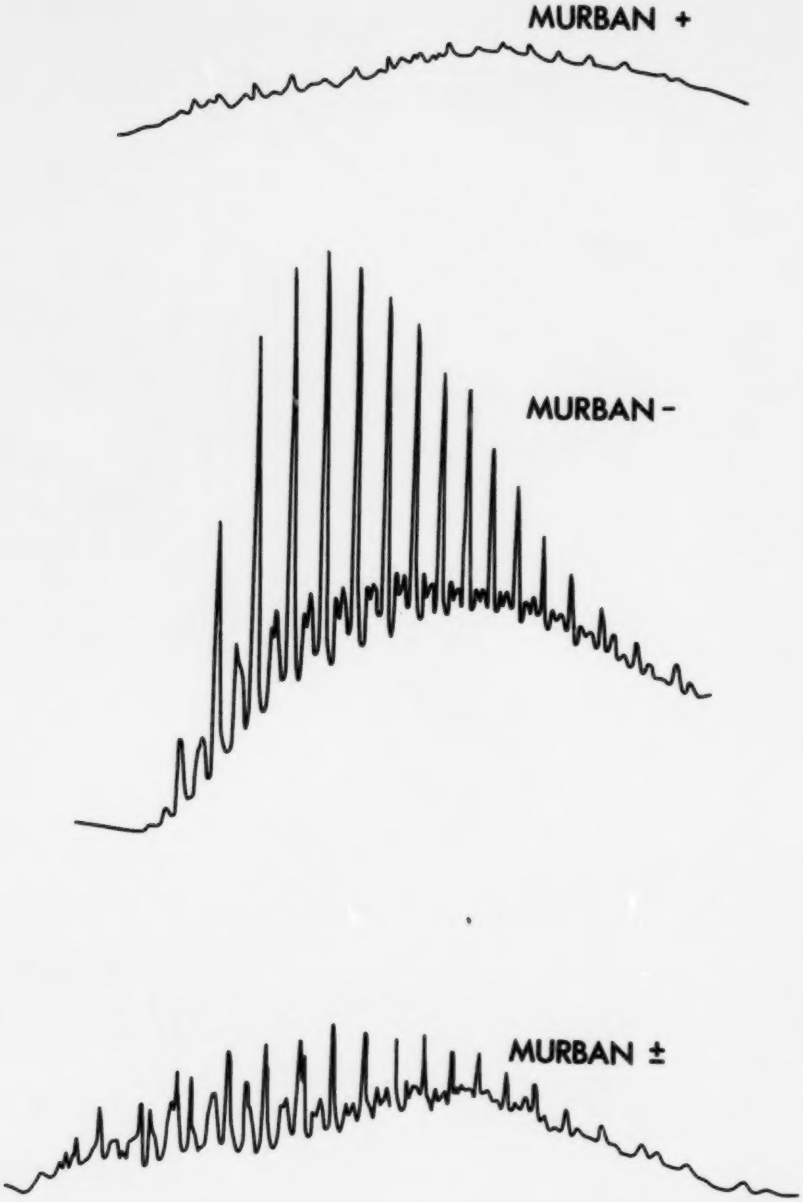

Figure 5. Typical G.C. profiles of saturate fraction of Murban oil before and after growth of enrichment populations (- = no degradation; ± = selective degradation; + = degraded profile).

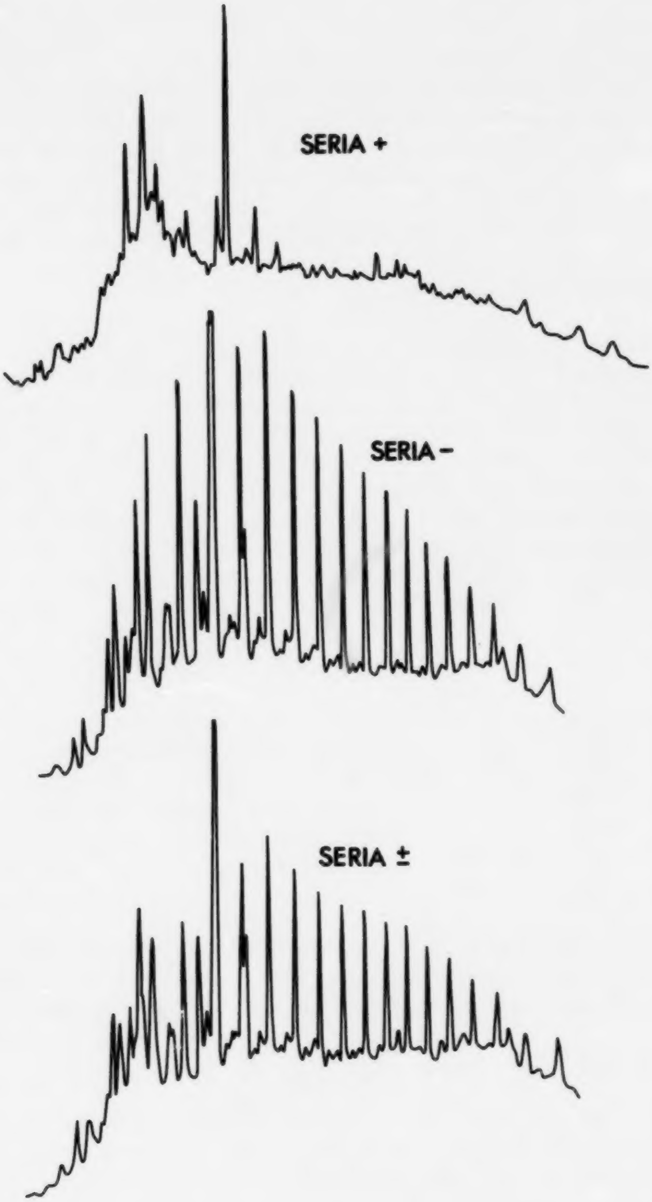

Figure 6. Typical G.C. profiles of saturate fraction of Seria oil before and after growth of enrichment populations (- = no degradation; ± = selective degradation; + = degraded profile).

exception that a saline basal agar was used for the growth of these marine isolates. One set was incubated at 20°C for 4 weeks and another set at 8°C for 8 weeks. The degree of oil degradation was evaluated by comparing the G.C. profile of the saturate fraction of recovered oil with that of undegraded (i.e. sterile control) Prudhoe Bay oil. The ability of selected fungi and yeasts to degrade Prudhoe Bay oil was also evaluated under highly aerobic conditions of the shake culture technique. All cultures were incubated at 20°C for 4 weeks before recovering the oil by pentane extraction and examining the status of the n-alkane and isoprenoid profile by gas chromatography.

Seaweeds and Cobbles

Samples of seaweeds were collected from various sites at different times throughout the study period using sterile scissors and forceps. Duplicate samples of approximately equal surface area of a given variety were collected. One sample was placed directly into a 500 mL, screw-capped Erlenmeyer flask containing 200 mL of sterile solution A supplemented with 2.0 mL of nitrogen and phosphorus solution and 0.2 mL of Prudhoe Bay oil. The other seaweed sample was placed in a plastic bottle containing 100 mL of sterile solution A and shaken "vigorously" 25 times. The seaweed was transferred to another bottle and the procedure repeated. The "washed" seaweed was then placed in a screw-capped Erlenmeyer flask containing a nitrogen and phosphorus supplement and Prudhoe Bay oil. The screw caps were replaced with sterile foam plugs prior to incubation under shaking conditions for 4 weeks at 8 C.

The presence of oil-degrading microorganisms on cobbles was determined by aseptically picking up cobbles (free of sand and weeds) on the beach and placing them in sterile 4L wide-mouth plastic jars containing 200 mL of sterile solution A. The jars and cobbles were shaken for 30 min after which the liquid was decanted into screw-capped 500 mL Erlenmeyer flasks and 2 mL of Prudhoe Bay oil were added to each flask. All flasks were incubated at 8°C with shaking for 4 weeks.

The residual oil was recovered from seaweed and cobble cultures using a pentane flotation technique and the status of the n-alkane and isoprenoid components of the saturate fraction determined by gas chromatography.

Gravimetric Studies

The rate of oil loss in water column samples by weathering and mineralization was determined using a gravimetric procedure. The loss of oil as a result of weathering (i.e. physical-chemical processes) was determined by monitoring as a function of incubation time the recovery of oil from 500 mL Erlenmeyer flasks containing 200 mL of sterile solution A and 0.4 g of Prudhoe Bay oil. The loss as a result of weathering and mineralization (i.e. physical-chemical and microbial action) was monitored in a similar manner except that the flasks contained 200 mL of water column material, 0.4 g of oil and 2.0 mL of a nitrogen and phosphorus solution. The difference in weight loss between the two series of flasks was considered to be a result of mineralization (i.e. microbial action).

The residual oil was recovered using chloroform which was subsequently evaporated at room temperature. The residue was extracted with benzene, transferred to a tared beaker, the benzene evaporated at room temperature and the residue weighed and reported as recovered oil. The chemical composition of the recovered oil was determined by extracting the residue from the benzene procedure with pentane. An aliquot of the pentane-soluble components was analyzed for residual n-alkanes and isoprenoids by gas chromatography. The remainder of the pentane soluble material was "topped" for 18 hr at 31°C in preparation for determining its chemical composition by a liquid chromatographic technique (13).

Gas Chromatography

Oil was recovered from cultures for gas chromatography by a pentane flotation technique. Cultures were cooled in an ice-water bath, and acidified with 50% HCl to pH $\leq$ 1 to minimize emulsion formation. Five mL of n-pentane were added and the culture flasks were stoppered with rubber bungs previously washed with pentane and benzene. The inner surfaces of the flasks were gently rinsed with the pentane layer and then the contents mixed for 25 min on a rotary shaker at 200 r.p.m. The oil/pentane mixture was recovered by replacing the solid stopper with one containing a short fine bore glass tube (3 mm internal diameter) and a larger bore glass tube (5 mm internal diameter) reaching the bottom of the flask. Tap water was slowly added via the larger tube so that the pentane/oil mixture slowly floated to the top of the Erlenmeyer flask and was recovered via the fine-bore tube using a microlitre hypodermic syringe. Samples which could not be analyzed immediately were acidified and stored at 4°C.

The n-alkane and isoprenoid content of the pentane-soluble fraction of recovered oil was determined by gas-liquid chromatography using a Varian (Model 1740) gas chromatograph equipped with flame ionization detectors and containing stainless steel columns (6.1 m x 0.32 cm) packed with 3% SE 30 Ultraphase on Chromosorb W (AW-DMCS), 80/100 mesh. Nitrogen was used as the carrier gas with a flow rate of 15 mL/min. The oven temperature was programmed from 100°C to 300°C at a rate of 10°/min and the upper temperature was held for 9 min. The injection ports and detectors were maintained at 325°C and 350°C, respectively.

Selected samples of aromatic fractions collected from the gravimetric analysis were analyzed by glass capillary gas chromatography. A Hewlett-Packard (Model 5710) gas chromatograph was modified to accept a capillary column as outlined (24,25). A 30 m x 0.25 mm WCOT SE54 (J & W Scientific Inc.) column was operated with helium as a carrier gas at a flow rate of 2 mL/min (split ratio 10:1). The initial column temperature of 100°C was held for 2 min and then programmed at a rate of 4°C/min to 270°C and held for 16 min. The injection port temperature was 300°C and the detector was 350°C.

The degradation of aromatic compounds also was studied by adding 200 ul Prudhoe Bay crude to replicate flasks containing 200 mL of sea water samples (some of which were supplemented with 2 mL nitrogen and phosphorus solution).

Sterile controls containing 200 mL artificial sea water plus oil were incubated on rotary shakers at 8°C. At various times, flasks were removed and acidified with 2 mL concentrated HCl. Prior to extraction, 100 µL of chrysene solution (2.08 mg/mL in methylene chloride) were added to each flask as a "recovery standard" and the flask was extracted with one 15 mL and two 10 mL portions of methylene chloride. The extracts were filtered through anhydrous Na_2SO_4, pooled, and methylene chloride added to a final volume of 40 mL. A 10 mL aliquot of this solution was concentrated to a volume of approximately 2 mL using a Kontes tube heater, appropriate concentrator tubes and reflux columns (Kontes Glass Co.). Two mL of hexane were added through the condenser and the sample was further concentrated to a final volume of 0.7 - 1.0 mL. This concentrate was fractionated (25) on a silica gel column prepared with n-pentane being substituted for petroleum ether. The sample was eluted with 5 mL n-pentane followed by 5 mL 20% methylene chloride in n-pentane and 17 mL methylene chloride. The first 17 mL of column effluent contained the saturate fraction and the final 10 mL contained the aromatic fraction. The eluant solvents were displaced, using a Kontes tube heater, with hexane and the saturates were concentrated to 0.7 to 0.9 mL and the aromatics concentrated to 0.6 to 0.75 mL. Fifty µL of a hexamethylbenzene standard were added to each aromatic sample. The saturates were made up with hexane to 1 mL and the aromatics to 0.8 mL total volume. These samples were resolved using the glass capillary column and conditions given above. However the temperature program was altered to 80°C for 4 min followed by an increase of 4°/min to 270°C which was held for 32 min.

Radiometric Procedures

The rate and extent of liberation of $^{14}CO_2$ from aliquots of Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-anthracene or [9-^{14}C]-phenanthrene were compared for representative beach, sub-tidal and water column samples. Twenty µL of "spiked" oil were added to 25 mL of water column samples or 1:3 dilutions of beach and sub-tidal samples in sterile solution A. The Erlenmeyer flasks had been modified (Figure 7) and a pleated filter paper was suspended in the plastic cup. Blanks were established by treating reaction mixtures at the initiation of the experiments with $HgCl_2$ at a rate of 100 µg/mL of liquid for beach and water column samples. Sub-tidal sediments (1:3 dilution) were heat sterilized. All flasks were incubated at 8°C with shaking. The amount of $^{14}CO_2$ liberated was determined by injecting a flask with 0.5 mL of 4N H_2SO_4 and adding 0.2 mL of CO_2-trapping agent (Carbo-Sorb II, Packard) to the filter paper suspended in the plastic cup and incubating with shaking for 60 min. The cup-filter paper assembly system was placed in scintillation vials containing 10 mL of Monophase-40 (Packard) and incubated for 24 hr at 6°C in the dark. The DPM's present were determined using a Mark III-6881-C-Analytical Liquid Scintillation Counter (Searle). Rates of hydrocarbon utilization were determined by periodically removing replicate flasks, counting the $^{14}CO_2$, and plotting the cumulative values. "Lag time" was defined as the time required for test values to exceed blank values by 5000 DPM. Rates of total specific hydrocarbons released as $^{14}CO_2$ per day per unit of inoculum were calculated after correcting for blanks and for changes in specific activities due to dilution by cold substrate present in Prudhoe Bay oil. Values

Figure 7. Type of Erlenmeyer flask used in radiometric studies.

(w/w%) of 0.45% for hexadecane and 0.1% for anthracene (based on laboratory data) and 2.0% for naphthalene and 2.0% for phenanthrene (26) in Prudhoe Bay oil were used in calculating dilutions of added radioactive compounds.

Microbial Numbers

Bacteria

The spread plate technique using a basal marine agar was used in all studies for determining total viable count. All dilution blanks contained artificial seawater salt solution at pH 7.3. Plates were dried before using by incubating for 2-3 days at 22°C and were stored at 4°C in sealed plastic bags. Plates used in the field were transported, inverted, in sealed plastic bags. Five replicate plates were prepared of each dilution and viable counts are reported as the average ± one standard deviation. Microbial counts from water column samples are reported per mL and beach and sediment data per g of dry material with the exception of the inter-tidal cores which were reported per g of wet weight.

Isolates for taxonomic studies were obtained by grouping colonies appearing on the 5 plates of the dilution used for enumeration as to colonial color and morphology. Colonies representative of each type were checked for purity by streaking on basal agar. Isolates were examined for cellular morphology, Gram reaction, presence of catalase and oxidase, and the utilization of glucose and lactose under aerobic and anaerobic conditions. The presence or absence of flagella (motility) of these isolates was determined either by examining negatively-stained specimens (1% phosphotungstic acid) by electron microscopy or by examining wet mounts with a light microscope. Bacteria were placed in genera based on the reactions to the above tests as outlined in Table 2. Type cultures of isolates were kept by storing at 4°C on basal marine agar on sealed agar slants. Some oil-degrading populations were maintained by monthly transfer in the enrichment medium containing Prudhoe Bay oil.

Fungi and Yeast

Fungal and yeast colony-forming units were enumerated using Sabouraud's agar plus antibiotics prepared in an artificial seawater solution (saline Sabouraud's agar). Three or 5 replicates of each dilution in artificial seawater solution were plated using the spread plate technique. Counts of colony forming units are reported as the average ± one standard deviation.

Fungal and yeast isolates were purified by repeated platings on saline Sabouraud's agar and pure cultures were stored on this agar at 4°C. Fungi were identified by the structure and color of colonies and their mycelium and spore-bearing hyphae when grown on malt agar. Fungi which could not be identified by these procedures were examined by the slide culture technique (see Appendix). Yeasts were tentatively classified on the basis of the colonial color and morphology when grown on saline Sabouraud's agar, their response to the API-20C (Analytical Products) sugar tests on their cellular morphology

TABLE 2. CHARACTERISTICS USED IN CLASSIFYING BACTERIAL ISOLATES

Genus	Gram Stain	Test[a]										
									Acid from			
		Rod(R) or Coccus (C)	Motile	Spores	Catalase	Oxidase	Growth		Glucose		Lactose	
							$+O_2$[b]	$-O_2$[b]	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Acinetobacter sp.	-	R	-	-	+	-	+	-	+/-	-	+/-	-
Aeromonas sp.	-	R/C	+/-	-	+	+	+	+	+	+	+/-	+/-
Alcaligenes sp.	-	R/C	+	-	+	+	+	-	-	-	-	-
Bacillus sp.	+	R	+/-	+	+/-	+	+	+/-	+/-	+/-	+/-	+/-
Chromobacterium sp.	-	R	+	-	+	+	+	+/-	+	+/-	-	-
corynebacteria	+	R	-	-	+	-	+	+	+	+	+	+
Cytophaga sp.	-	R	+	-	+/-	+	+	+/-	+/-	+/-	+/-	+/-
Flavobacerium sp.	-	R	+/-	-	+	+	+	+/-	+/-	+/-	+/-	+/-
Pseudomonas sp.	-	R	+	-	+	+	+	-	+	-	-	-
Vibrio sp.	-	R	+	-	+	+	+	+	+	+	+/-	+/-

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[a] Characteristics used for primary computer classification. Some isolates required the use of additional information and Bergey's Manual of Determinative Bacteriology, 8th Ed.

[b] $+O_2$ = aerobic; $-O_2$ = anaerobic

on malt agar and presence of spores as determined by light microscopy.

Sub-tidal Samples (Battelle Institute, Environmental Protection Agency - National Oceanic and Atmospheric Administration)

Samples were supplied by the Battelle Institute (Sequim) frozen at -20°C and they were maintained at this temperature until analyzed.

Samples were thawed overnight at 4°C and 10-fold dilutions of cores were prepared in sterile solution A. These dilutions were used to inoculate test tubes containing one of three different media. To enumerate oil-degrading heterotrophs, diluted samples were inoculated into test tubes containing 5 mL of solution A, 0.05 mL of nitrogen and phosphorus solution and 0.05 mL of Prudhoe Bay oil. Total heterotrophs were estimated using 5 mL of a medium enriched with amino acids, and the number of autotrophs determined using a medium containing 5 mL of solution A. All dilutions of samples were set up in quintuplicate and incubated at 20-22°C for 4 weeks. Growth was scored as plus or minus and statistical tables were used to calculate the Most Probable Number (MPN) of bacteria present. Data are reported as MPN/g wet weight.

Physical and Chemical Measurements

Physical Analyses

All instruments were calibrated before and after each sample trip in addition to the usual adjustments made prior to each measurement.

The pH of water column samples was determined using a portable Radiometer pH meter (Model #29) which was standardized using a Radiometer (51001) buffer system (pH 7.0). Salinity and water column temperature were measured in the field using a Yellow Springs SCT meter (Model #33). The salinity function of this instrument was calibrated according to the manufacturer's specifications (i.e. with a standard solution of KCl in distilled water). Beach temperatures were obtained using a standard laboratory thermometer (0-50°C scale). Dissolved oxygen content of water column samples was determined on site using a Yellow Springs Oxygen meter (Model #52) which was calibrated against a dropping mercury electrode system at the City of Edmonton's Goldbar Waste Water Treatment Plant analytical control laboratory.

Visual observations of habitat parameters were recorded and submitted as part of the computer data bank but are not included in this report.

The dry weights of beach and sediment samples were determined by accurately weighing, in triplicate, 1 to 2 g samples and drying for 72 h at 100°C. The average difference between the wet and dry weights was used to calculate a correction factor for reporting results on a dry weight basis. The weight of suspended material in water column samples was obtained by filtering aliquots through glass fibre filters (Reeve Angel) and weighing after drying to constant weight at 104°C.

Chemical Analyses

Water samples taken for grease and oil analysis were collected in wide-mouth, one-quart glass jars fitted with teflon-lined caps. Each container was rinsed with carbon tetrachloride, and allowed to dry prior to sampling. The samples were preserved by adding 50% H_2SO_4 (to pH<2) and stored at 4°C until analyzed. Standard Methods (27), Method 502B, was used for the analysis with carbon tetrachloride rather than using freon for extraction.

In all cases, sand samples were collected in wide-mouth plastic bottles and were stored frozen (-20°C) until they were analyzed. Grease and oil analyses of beach samples were done using the Soxhlet extraction method with freon [Standard Methods (27), Method 502D]. Grease and oil concentrations were measured spectrophotometrically using a Perkin-Elmer 297 IR spectrophotometer with 50 mm quartz cells.

A 10 mL aliquot of the preserved water sample for grease and oil analysis was used for total organic carbon analysis. Inorganic carbon was removed from the acidified sample by sparging with CO_2-free N_2 as outlined in Standard Methods (27), Method 505. Carbon analyses were performed using a Beckman 915A Total Organic Carbon Analyzer.

Water samples for ammonia-nitrogen and nitrate-nitrogen analyses were collected in plastic bottles and acidified to pH<2 with 50% H_2SO_4. Ammonia-nitrogen was determined by the distillation method using a 50 mL sample in a micro-Kjeldahl apparatus, followed by Nesslerization [Standard Methods (27), Method 418B]. The brucine method [Standard Methods (27), Method 419D] was used for nitrate-nitrogen analysis of water samples.

The amount of ammonia-nitrogen present in beach samples was determined by placing an accurately known amount of beach material (approximately 2 g wet weight) into a micro-Kjeldahl flask, then adding 10 mL 2N KCl and 0.1 g MgO (28). The ammonia was steam distilled, trapped and determined by Nesslerization. Nitrate-nitrogen was extracted from accurately known weights of sand (approximately 10 g wet weight) with 50 mL 0.015 M $CaSO_4$ and vigorous shaking for 10 min (28). The extract was filtered through Whatman 42 paper and nitrate-nitrogen determined by the brucine method given above.

Water samples were analyzed for total orthophosphate using the ascorbic acid method [Standard Methods (27), Method 425F]. These samples were collected in plastic bottles and kept on ice during transport and stored frozen in the laboratory until analyzed. Available orthophosphate was extracted from 15 g of sand dried at 105°C for 24 h, with 75 mL 0.03 N NH_4F in 0.03 N H_2SO_4. After 2 min shaking, the extract was filtered through Whatman 30 filter paper and a 50 mL aliquot was analyzed for orthophosphate using the ascorbic acid method.

Data Management

All the data collected or obtained by the physical-chemical and microbiological methods used in this study together with the characteristics of sample sites and types are available from the N.O.A.A., Washington, D.C., National Oceanographic Data Center.

SECTION 5

RESULTS

Survey for Oil-Degrading Activity

Water Column and Beach Samples

The state of biodegradation of recovered Prudhoe Bay oil, as shown by the G.C. profiles of the saturate fraction, after 28 days incubation at 8°C with water column and beach samples supplemented with nitrogen and phosphorus, is presented in Tables 3 and 4. The extent of n-alkane and isoprenoid utilization varied both with sample site and sampling time. The samples taken in the fall showed the greatest oil-degrading capability. No degradation of these Prudhoe Bay oil fractions was observed in a series of similar experiments which did not receive a supplement of nitrogen and phosphorus.

Table 5 is a summary of the oil-degrading capability of sites which were sampled over a 2 year period. Greater seasonal variation in oil-degrading ability as determined by "Degradative Capacity Index" is noted in water column samples, and in particular in those samples from "pristine" areas (e.g. Pt. Partridge). In contrast, beach samples from areas more likely to have contact with hydrocarbons (e.g. E. Fidalgo) show a much more consistent oil-degrading capability as a function of season. Samples taken between April and September show a slightly higher degradative capability (Table 5) for both beach and water column samples than those taken between October and March (ratios of 2.2:1.6 for beach and 2.0:1.4 for water column).

The "Degradative Capacity Indices" for all sites sampled during the current study period (fall of 1978-1979) are presented in Table 6. As previously noted, samples taken from areas more likely to be subjected to oil or hydrocarbon exposure (e.g. Pt. Angeles harbor area) show a greater ability to degrade Prudhoe Bay oil than do samples taken from a "pristine" area (e.g. Dungeness spit area). This is clearly shown by the data presented in Table 7 where the "Degradative Capacity Index" for water and beach samples from the Pt. Angeles area is summarized. The area west of Morse Creek to the Ediz Hook Pilot Station has a much greater capability to degrade Prudhoe Bay oil than the area east of Morse Creek to the outer Dungeness Spit navigational markers.

Inter-tidal Samples

The oil-degrading ability of inter-tidal core samples is presented in Table 8. Only the surface cm of the core taken in Pt. Angeles harbor at Peabody Creek was able to completely utilize the n-alkanes and isoprenoids present in Prudhoe Bay oil. The microorganisms present in the rest of the material in this core were relatively ineffective in bringing about changes in the n-alkane portion of this oil. In contrast, the oil-degrading activity

TABLE 3. G.C. PROFILE OF PENTANE EXTRACT OF RECOVERED PRUDHOE BAY OIL AFTER 28 DAYS INCUBATION AT 8°C WITH WATER COLUMN SAMPLES SUPPLEMENTED WITH NITROGEN AND PHOSPHORUS.

Site	G.C. Profile[a]					
	1978			1979		
	Aug. 22	Oct. 1	Nov. 21	Jan. 14	Apr. 2	June 18
Samish Island	S	-	N	-	S	-
E. Fidalgo	P	-	P	-	S	-
Pt. Partridge	I	-	S	-	S	-
Freshwater Bay	-[b]	S	-	I	-	-
Pt. Angeles - Ediz Hook #1	-	S	-	S	-	P
Pt. Angeles - Ediz Hook #2	-	C	-	S	-	S
Pt. Angeles - Southside #1	-	C	-	S	-	S
Pt. Angeles - Southside #2	-	C	-	C	-	I
Pt. Angeles - Southside #3	-	C	-	S	-	N
West of Morse Creek	-	S	-	S	-	S
West of Green Pt. (near cavern)	-	N	-	-	-	S
Green Pt. #1	-	S	-	-	-	S
Green Pt. #2	-	S	-	-	-	-
Green Pt. #3	-	S	-	N	-	S
Dungeness #1	-	N	-	S	-	N
Dungeness #2	-	N	-	S	-	S
Dungeness Lagoon	-	-	-	-	-	N

[a] N = no degradation; S = selective utilization of n-alkanes C_{12} to C_{19}; P = partial removal, n-alkane peaks still discernible; I = only isoprenoid peaks remaining; C = complete utilization of n-alkanes and isoprenoids.

[b] not sampled

TABLE 4. G.C. PROFILE OF PENTANE EXTRACT OF RECOVERED PRUDHOE BAY OIL AFTER 28 DAYS INCUBATION AT 8°C WITH BEACH SAMPLES SUPPLEMENTED WITH NITROGEN AND PHOSPHORUS.

Site	G.C. Profile[a]					
	1978			1979		
	Aug. 22	Oct. 1	Nov. 21	Jan. 14	Apr. 2	June 18
Samish Island	C	-	S	-	S	-
E. Fidalgo	I	-	I	-	P	-
Pt. Partridge	N	-	N	-	S	-
Freshwater Bay (Western edge)	-[b]	I	-	I	-	S
Pt. Angeles - Ediz Hook #1	-	I	-	C	-	I
Pt. Angeles - Ediz Hook #2	-	I	-	I	-	S
Pt. Angeles - Southside #1	-	I	-	-	-	S
Pt. Angeles - Southside #2	-	I	-	I	-	P
Pt. Angeles - Southside #3	-	C	-	I	-	P
West of Morse Creek	-	I	-	S	-	-
West of Green Pt. (near cavern)	-	I	-	-	-	S
Green Pt. #1	-	N	-	-	-	-
Green Pt. #2	-	S	-	-	-	-
Green Pt. #3	-	N	-	P	-	S
Dungeness #1	-	S	-	S	-	N
Dungeness #2	-	P	-	N	-	N
Dungeness Lagoon	-	-	-	-	-	S

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[a] N = no degradation; S = selective utilization of n-alkanes C_{12} to C_{19}; P = partial removal, n-alkane peaks still discernible; I = only isoprenoid peaks remaining; C = complete utilization of n-alkanes and isoprenoids.

[b] not sampled

TABLE 5. SUMMARY OF OIL-DEGRADING ACTIVITY[a] AT SITES SAMPLED FROM 1977 - 1979.

	Date		Samish Island	East Fidalgo	Pt. Partridge	Pt. Angeles Southside #3
Water Column	1977	April 16	I	C	S	P
		Sept. 19	I	I	I	P
	1978	Jan. 8	N	S	S	I
		Aug. 22	S	P	I	-
		Oct. 1	-[b]	-	-	C
		Nov. 21	N	P	S	-
	1979	Jan. 14	-	-	-	S
		April 2	S	S	S	-
		June 18	-	-	-	N
Degradative Capacity Index			1.3	2.2	1.7	2.0
Beach	1977	April 16	S	C	I	I
		Sept. 19	P	I	P	I
	1978	Jan. 8	S	C	S	I
		Aug. 22	C	I	N	-
		Oct. 1	-	-	-	C
		Nov. 21	S	I	N	-
	1979	Jan. 14	-	-	-	I
		April 2	S	P	S	-
		June 18	-	-	-	P
Degradative Capacity Index			1.7	3.2	1.2	3.0

[a] N = no degradation; S = selective utilization, C_{12} to C_{19};
P = partial removal, n-alkane peaks still discernible;
I = only isoprenoid peaks remaining; C = complete utilization of n-alkanes and isoprenoids

[b] = not sampled

TABLE 6. DEGRADATIVE CAPACITY INDEX FOR SITES SAMPLED 1978 - 1979[a].

Site	Degradative Capacity Index		
	Beach	Water Column	Combined[b]
Samish Island	2.0	0.7	1.3
E. Fidalgo	2.7	1.7	2.2
Pt. Partridge	0.3	1.7	1.0
Freshwater Bay	2.3	2.0	2.2
Pt. Angeles-Ediz Hook #1	3.3	1.3	2.3
Pt. Angeles-Ediz Hook #2	2.3	2.0	2.2
Pt. Angeles-Southside #1	2.0	2.0	2.0
Pt. Angeles-Southside #2	2.7	3.7	3.3
Pt. Angeles-Southside #3	3.0	1.7	2.3
West of Morse Creek	2.0	1.0	1.5
West of Green Point (near cavern)	2.0	0.5	1.3
Green Point #1	0	1.0	0.5
Green Point #2	1.0	1.0	1.0
Green Point #3	1.0	0.7	0.8
Dungeness #1	0.7	0.3	0.5
Dungeness #2	0.7	0.7	0.7
Dungeness Lagoon	1.0	0	0.5

[a] 28 days incubation at 8°C supplemented with nitrogen and phosphorus

[b] average value of beach plus water column indices

TABLE 7. DEGRADATIVE CAPACITY INDEX FOR PT. ANGELES SAMPLES SITES[a] (1978 - 1979).

Sample	Nitrogen Phosphorus Supplementation	Degradative Capacity Index		
			1979	
		Oct 1/1978	Jan 14	June 18
All Sample Sites				
Water	+	1.7	1.4	1.0
	-	0	0	0
Beach	+	2.2	2.2	1.2
	-	0	0	0
Area West of Morse Creek to Ediz Hook Pilot Station				
Water	+	2.7	1.5	1.7
	-	0	0	0
Beach	+	3.2	2.8	1.8
	-	0	0	0
Area East of Morse Creek to Outer Dungeness Navigational Marker				
Water	+	0.5	0.7	0.8
	-	0	0	0
Beach	+	1.2	1.0	0.5
	-	0	0	0

[a] 28 days incubation at 8°C

TABLE 8. OIL-DEGRADING CAPABILITY[a] OF INTER-TIDAL CORE SAMPLES.

				Bacterial Counts			
				initial[b]		final[c]	
Site	Depth (cm)	Description	G.C. Profile	number per gm	% pigmented	number per ml	% pigmented
Pt. Angeles (Southside, site 27, Peabody Creek; June 1979)	0-1	coarse sand	C	$2.0(\pm0.4)x10^{7}$	40	$1.8(\pm0.2)x10^{8}$	55
	2-4	coarse sand & small gravel	S	-[d]	-	-	-
	4-6	small gravel	S	-	-	-	-
	6-8	small gravel & decaying plant material	S	-	-	-	-
	8-10	small gravel & decaying plant material	S	-	-	-	-
	10-12	decaying plant material	S	-	-	-	-
	12-14	clay & muck	S	$4.2(\pm0.5)x10^{5}$	7	$6.8(\pm0.7)x10^{7}$	14
E. Fidalgo (site 6, October 1979)	1-2	reddish brown medium size sand particles	P/I	$4.2(\pm1.3)x10^{6}$	45	$6.9(\pm0.6)x10^{7}$	12
	16-18	reddish brown medium size sand particles	P/I	-	-	$3.5(\pm0.8)x10^{8}$	35
	32-34	red sand with clay particles	P/I	$3.7(\pm0.4)x10^{4}$	12	$1.2(\pm0.1)x10^{8}$	5

TABLE 8. continued ....

Site	Depth (cm)	Description	G.C. Profile	Bacterial Counts			
				initial[b]		final[c]	
				number per gm	% pigmented	number per ml	% pigmented
Dungeness Spit (site 33, October 1979)	1-2	grey; coarse sand and pebbles	S	$< 10^4$	-	$5.3(\pm0.5)\times10^7$	50
	10-12	grey; coarse sand and pebbles	N/S	-	-	$7.8(\pm1.2)\times10^7$	30
	20-22	grey; coarse sand and pebbles	N/S	$4.9(\pm0.9)\times10^4$	30	$5.3(\pm0.6)\times10^7$	5

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[a] incubated with nitrogen and phosphorus at 8°C for 28 days

[b] bacterial colony forming units in original samples

[c] bacterial colony forming units in enrichment cultures

[d] samples not analyzed

present in the E. Fidalgo core, while only bringing about the partial modification of the n-alkanes, is uniformly distributed throughout the core. The two-fold reduction in bacterial colony forming units between the surface and bottom of this core has not affected the oil-degrading activity present. Very little oil-degrading activity is demonstrated in the material recovered in the Dungeness core although the bacterial numbers detected are similar to those found in the bottom of the E. Fidalgo core. The difference noted in the proportion of pigmented colonies in the samples indicates the presence of different bacterial types in the populations. However such differences are not related to the oil-degrading capability of such populations.

Seaweeds

The results in Table 9 show that very little change was brought about by microorganisms associated with seaweeds during the initial 16 day incubation period. However the presence of oil-degrading microorganisms is indicated as some of the transfers after 28 days incubation did bring about major changes in the chemical composition of Prudhoe Bay oil. The data in this Table also indicate that increasing the amount of nitrogen and phosphorus added was detrimental to oil degradation.

The oil-degrading capability of "washed" and "unwashed" seaweed samples is compared in the data presented in Table 10. If an oil-degrading microbial flora is present on seaweed the "washing" technique did not remove it. In fact the data suggest that a greater degree of oil degradation occurs with "washed" than with "unwashed" seaweed.

Cobbles

The oil-degrading activity of microorganisms released from cobbles by "washing" is presented in Table 11. Samples taken from cobbles originating from areas more likely to have been exposed to hydrocarbon or oil spillage (e.g. Pt. Angeles harbor (Ediz Hook #2) and E. Fidalgo) result in a greater change in the chemical composition of Prudhoe Bay oil than samples from more "pristine" environments (e.g. Pt. Partridge). Transfer from initial enrichments, as for seaweed samples, showed better degradation than was observed in the initial enrichments.

TABLE 9. UTILIZATION OF OIL BY BACTERIA ATTACHED TO SEAWEEDS (COLLECTED AUGUST 1978).

	G.C. Profile			
	Initial Enrichment[a]		Transfer[b]	
	I[c]	II[d]	I	II
Kelp	N	N	N	N
Bleached Kelp	N/S	N	I	N
Eel Grass	N	N	I	I
"Cedar-like" leaves	N/S	N	I	I
"Lettuce"	N	N	I	N

[a] 16 days incubation at 8°C

[b] 28 days incubation at 8°C; 5% inoculum

[c] regular concentration of nitrogen and phosphorus

[d] twice the concentration used in "c"

TABLE 10. UTILIZATION OF OIL BY "WASHED" AND "UNWASHED" SEAWEED (COLLECTED AUGUST AND OCTOBER 1979).

Site	Type	~Surface Area (cm^2)	Washed/Unwashed	G.C. Profile[a] Enrichment	G.C. Profile[a] Transfer
(A) AUGUST 1979					
Pt. Partridge	green-brown seaweed	10	W	N	N
			U	N	N
	green-brown seaweed	50	W	N	N
			U	N	N
	green "lettuce"	16	W	N	S
			U	N	N
	green "lettuce"	16	W	P	-[b]
			U	N	-
(B) OCTOBER 1979					
Peabody	green-brown seaweed	40	W	S/P	-
			U	S	-
Pt. Partridge	Kelp (dead)	30	W	S/P	-
			U	S	-
E. Fidalgo	Eel grass	40	W	I	-
			U	S	-

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[a] 28 days incubation at 8°C supplemented with nitrogen and phosphorus

[b] not done

TABLE 11. UTILIZATION OF PRUDHOE BAY OIL BY BACTERIA ATTACHED TO COBBLES.

Sample				G.C. Profile		
Site	Condition	Tide Level	Surface Area (cm^2)	Enrichment[a]	Transfer[b]	Enrichment Bacterial Count/ml
(A) JANUARY 1979						
Pt. Angeles Ediz Hook #2	wet	low	51.8	S	I	$1.2(\pm 0.2) \times 10^8$
	damp		60.4	P	I	$1.1(\pm 0.2) \times 10^8$
	dry	high	50.3	S	I	$4.5(\pm 1.3) \times 10^7$
Pt. Partridge	wet	low	106.4	N	I	$1.8(\pm 0.1) \times 10^7$
	damp		93.0	N	I	$7.4(\pm 1.4) \times 10^7$
	dry	high	72.4	N	N	$2.2(\pm 0.4) \times 10^6$
(B) APRIL 1979						
E. Fidalgo	wet	low	44.5	S	I	$1.3(\pm 0.1) \times 10^8$
	damp		48.0	S	P	$2.8(\pm 0.6) \times 10^8$
	dry	high	47.2	P	P	$1.7(\pm 0.1) \times 10^8$
Pt. Partridge	wet	low	35.6	N	N	$1.4(\pm 0.1) \times 10^7$
	damp		35.8	P	N	$8.0(\pm 0.1) \times 10^7$
	dry	high	55.1	N	N	$1.1(\pm 0.1) \times 10^6$

[a] 4 weeks incubation at 8°C supplemented with nitrogen and phosphorus

[b] inoculum 5% v/v of original enrichment; 4 weeks incubation at 8°C

Rate of Oil Utilization

Gravimetric Studies

The rate of weight loss of Prudhoe Bay oil was determined after incubation with water column samples (April, 1979) from E. Fidalgo and Pt. Partridge supplemented with nitrogen and phosphorus. Changes in the weight of recoverable oil were observed only with the E. Fidalgo water samples and these are presented in Figure 8 and Table 12. The maximum rate of mineralization of oil observed with the E. Fidalgo water column samples was 50.5 mg/L/day and occurred during the 28th to 38th day of incubation. The changes in the weight percentage recoveries of the asphaltene, saturate, aromatic and N,S,O components of the oil recovered from the E. Fidalgo experiments are presented in Table 12 and Figure 9. The changes resulting from the incubations of oil with Pt. Partridge water and a control series are also presented in Figure 9. Very little change was observed between the recovery patterns of the components of Prudhoe Bay oil between the control and the Pt. Partridge sample. However the saturate and aromatic components in the E. Fidalgo series start to decrease between the 20th and 25th day of incubation. This decrease terminated between the 35th and 40th day of incubation. The slightly increased level of these components observed after the 40th day of incubation is a result of recovery problems incurred by the formation of very stable oil/water emulsions by cultures during the latter part of the incubation period. Data on the G.C. profile of the saturate fraction of oil recovered from the E. Fidalgo series of cultures (Table 12) indicate a rapid utilization of the n-alkanes and isoprenoids during the 20th to the 35th days of incubation. The status of the G.C. profile of recovered oil is also presented in Table 12.

The results of similar studies using water column samples from the Pt. Angeles area (June, 1979; Ediz Hook, Peabody Creek and Dungeness Spit #2) are presented in Figures 10 and 11. The data on weight loss (Figure 10 and Tables 13, 14, and 15) indicate a more rapid removal of oil by microorganisms present in the water column material from Pt. Angeles harbor (Peabody Creek site). The longest lag, 40 days, (before oil was degraded) occurred with water samples from Ediz Hook. Water samples from Peabody Creek and Dungeness area initiated oil degradation after approximately 22 and 28 days incubation respectively. Greater variation between samples also is observed with water samples from the more "pristine" areas(e.g. Ediz Hook and Dungeness Spit) than from more "polluted" areas(e.g. Peabody Creek). The maximum rates of removal of oil were calculated to be 37.7, 14.6 and 13.5 mg/L/day for water column samples from Peabody Creek, Dungeness and Ediz Hook respectively. Changes in the four major components of Prudhoe Bay oil are presented in Figure 11, and indicate (as have other studies) that the saturate and aromatic fractions are readily metabolized with lags similar to those shown in Figure 9 for weight loss of oil. The status of the G.C. profile of recovered oil is also presented in Tables 13, 14, and 15 for Ediz Hook, Peabody Creek and Dungeness incubations respectively.

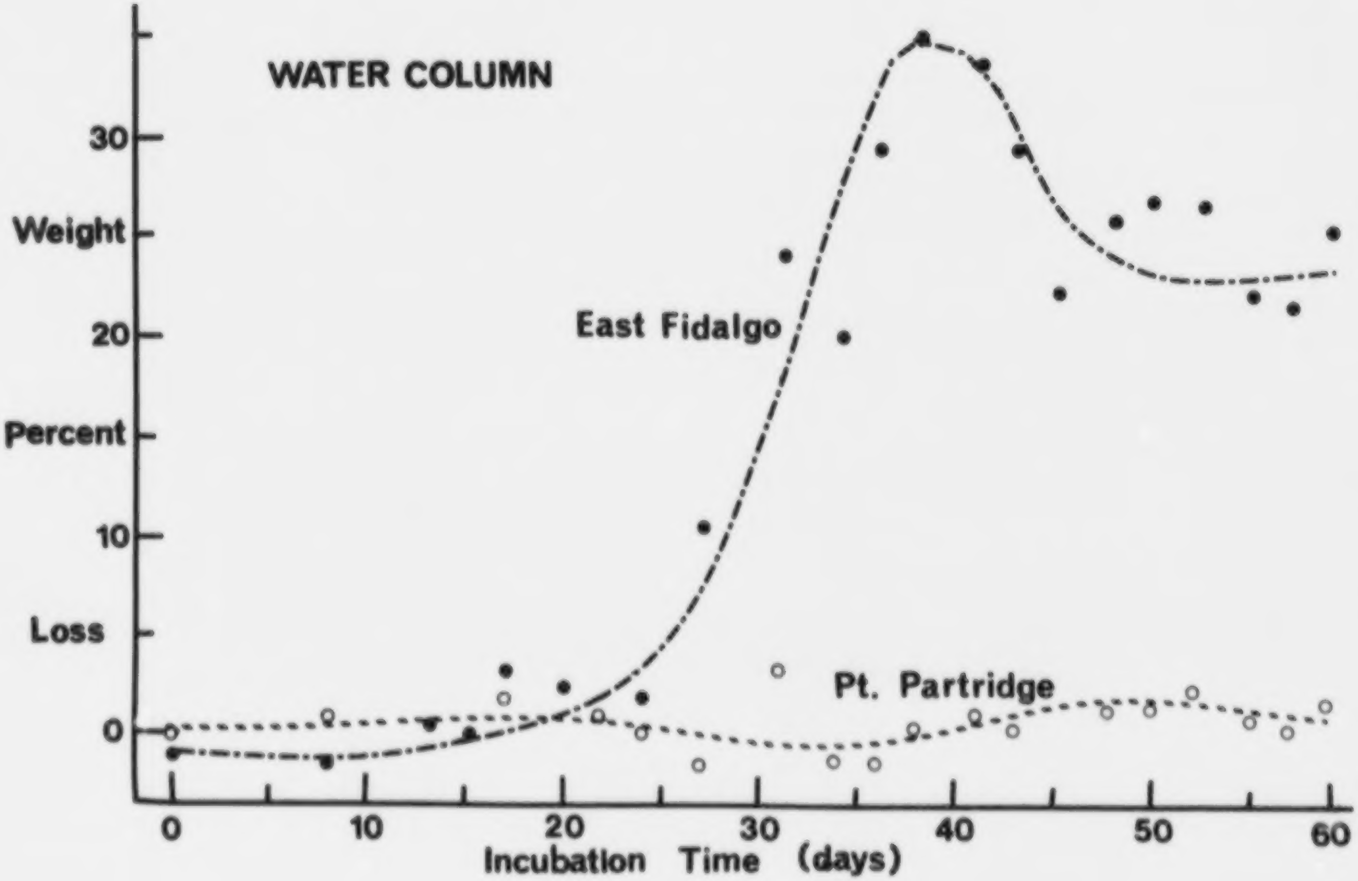

Figure 8. Weight percent loss of Prudhoe Bay oil as a result of incubation (8°C) with water column samples from E. Fidalgo and Pt. Partridge (April, 1979).

TABLE 12. GRAVIMETRIC AND G.C. CHANGES IN PRUDHOE BAY OIL BROUGHT ABOUT BY INCUBATION WITH WATER COLUMN SAMPLES FROM E. FIDALGO.

Incubation Time(Days)	G.C.[a] Profile	% Weight Loss of Oil	Corrected[b] % Weight Loss	% Composition of Residual Oil[c]			
				Saturates	Aromatics	Asphaltenes	NSO's
0	N	28.9	-1.29	25.9	25.2	10.0	10.4
8	N	25.3	-1.47	25.3	23.9	11.3	11.8
13	N	27.9	0.23	25.1	23.4	10.6	12.8
15	N	26.9	-0.12	25.9	24.5	10.9	11.4
17	N	28.5	3.14	25.3	22.9	11.0	11.6
20	N	27.9	2.22	25.3	22.5	11.6	12.1
22	S	27.9	1.87	25.1	23.3	11.9	11.9
24	S	27.8	1.67	24.8	23.0	11.9	12.3
27	I	34.4	10.28	20.8	21.9	11.4	12.2
29	P	31.1	6.66	23.4	22.7	7.1	15.9
31	I	46.7	24.03	16.0	17.3	11.3	10.4
34	C	46.2	19.84	14.9	18.9	10.7	10.6
36	C	53.5	29.42	12.1	16.6	9.5	10.1
38	C	57.8	34.81	10.7	15.1	8.8	10.0
41	C	53.2	33.61	11.0	16.6	10.3	9.9
43	C	50.1	29.37	12.2	17.4	11.0	10.4
45	C	45.1	22.11	13.6	18.9	12.7	11.0
48	C	48.4	25.62	12.6	17.9	12.8	10.6
50	C	47.4	26.90	13.5	19.3	12.4	9.9
52	C	48.4	26.36	12.7	17.9	12.9	10.6
55	C	45.3	21.85	14.1	19.0	12.8	10.8
57	C	45.3	21.69	13.1	18.4	12.3	13.4
59	C	48.6	25.36	12.3	16.7	12.0	12.6

[a] N = no degradation; S = selective utilization of n-alkanes C_{12} to C_{19}; P = partial removal, n-alkane peaks still discernible; I = only isoprenoid peaks remaining; C = complete utilization of n-alkanes and isoprenoids.

[b] Corrected by subtracting respective uninoculated control values

[c] The %'s of each oil fraction were calculated based on the initial wt. of oil present in each flask and not as a percentage of the residual weight.

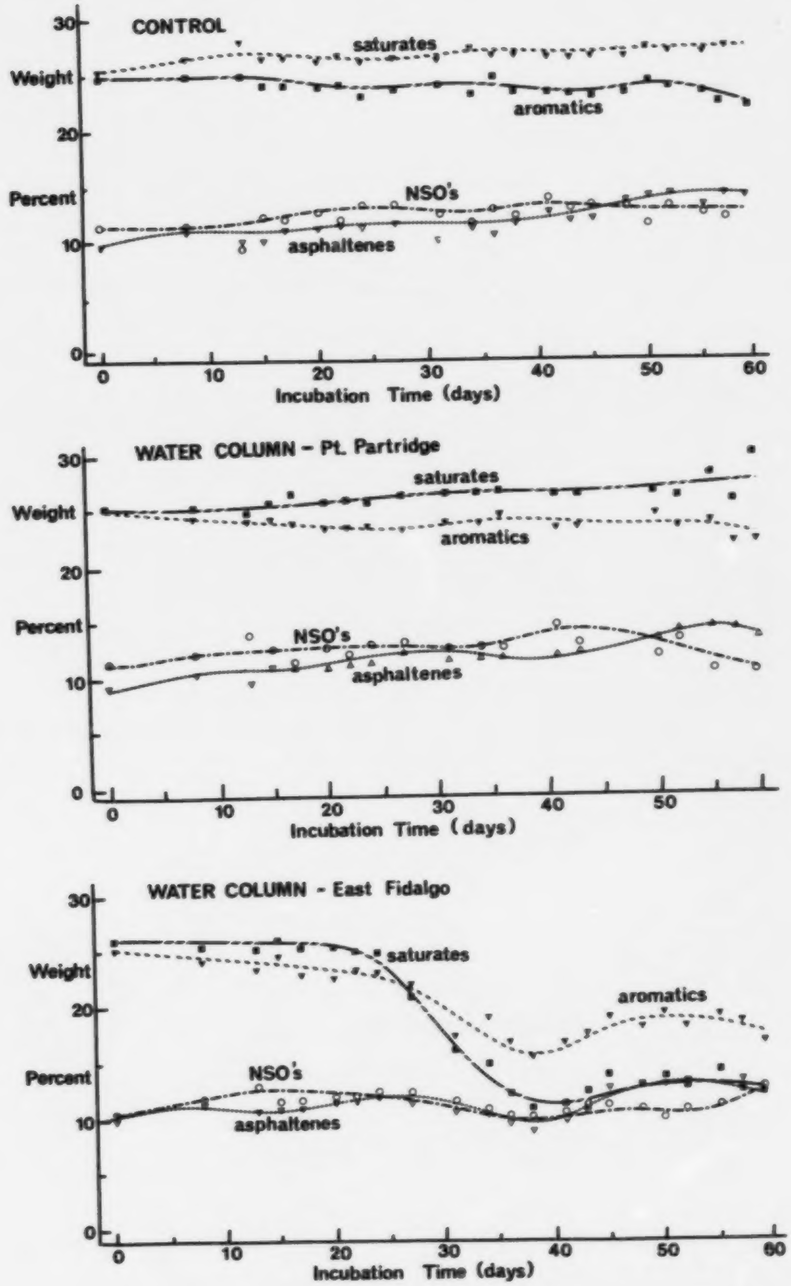

Figure 9. Changes in the components of Prudhoe Bay oil as a result of incubation with water column samples from northern Puget Sound (April, 1979).

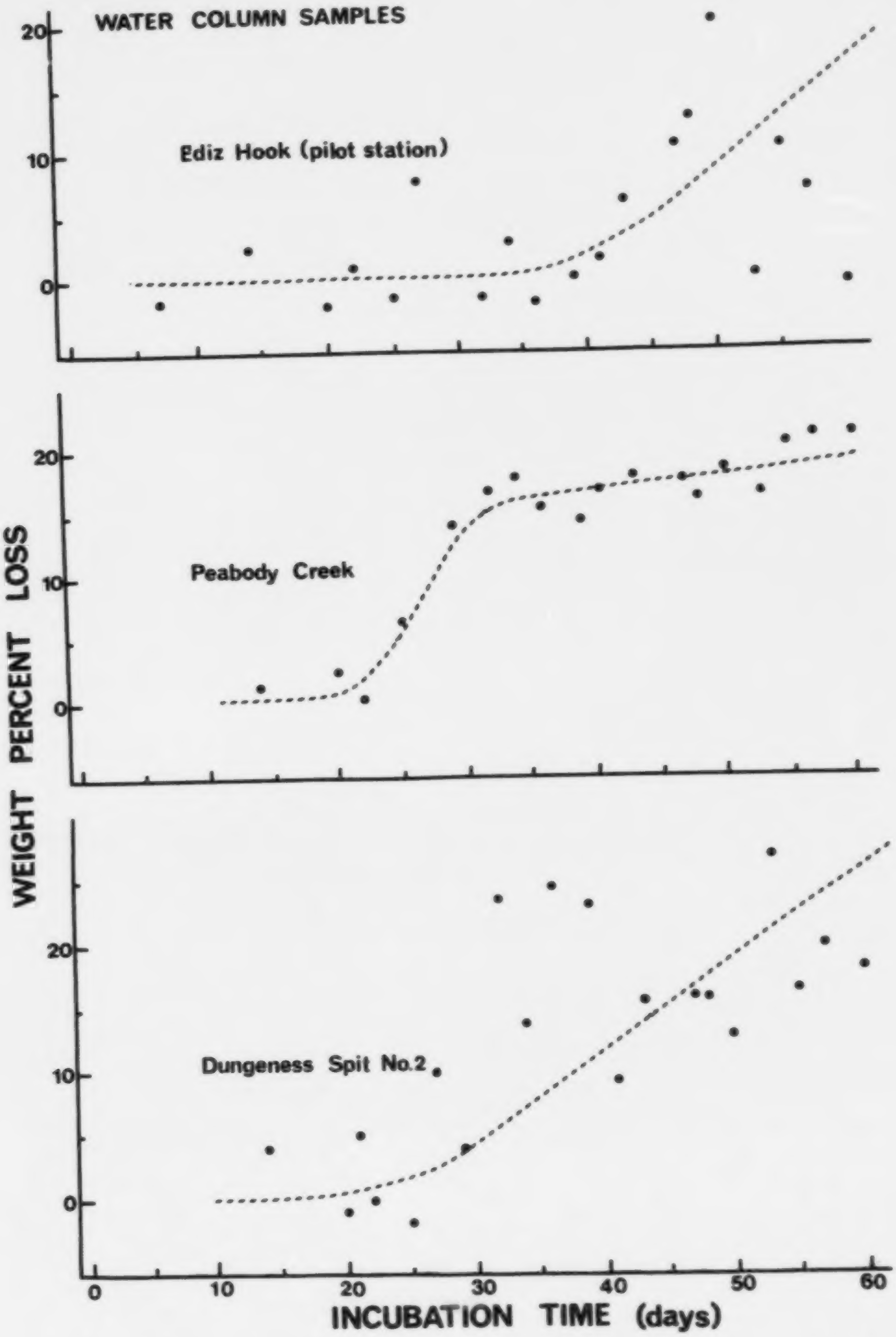

Figure 10. Weight percent loss of Prudhoe Bay oil as a result of incubation (8°C) with water column samples from the Pt. Angeles area (June, 1979).

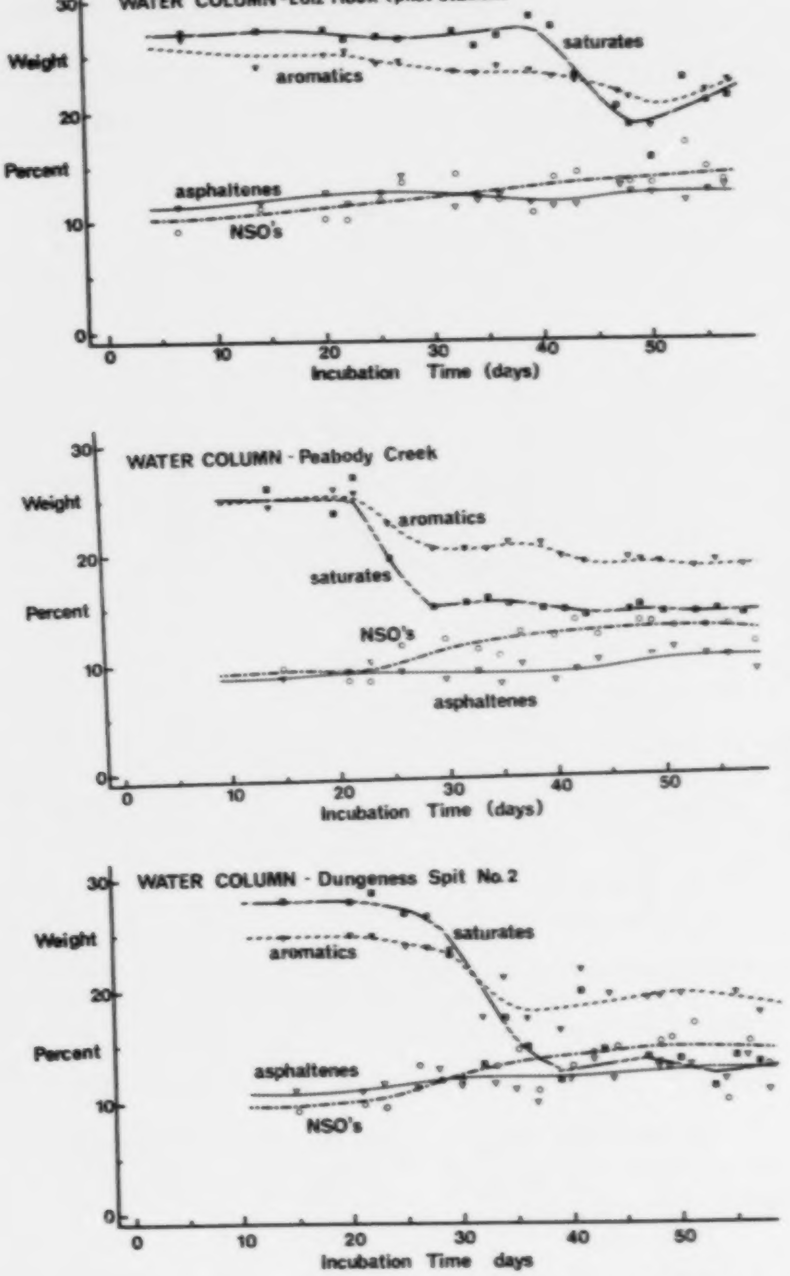

Figure 11. Changes in the components of Prudhoe Bay oil as a result of incubation (8°C) with water column samples from the Pt. Angeles area (June, 1979).

TABLE 13. GRAVIMETRIC AND G.C. CHANGES IN PRUDHOE BAY OIL BROUGHT ABOUT BY INCUBATION WITH WATER COLUMN SAMPLES FROM PT. ANGELES HARBOR (EDIZ HOOK, JUNE, 1979).

Incubation Time(Days)	G.C.[a] Profile	% Weight Loss of Oil	Corrected[b] % Weight Loss	% Composition of Residual Oil[c]			
				Saturates	Aromatics	Asphaltenes	NSO's
7	N	28.46	-1.93	27.10	26.81	11.32	9.08
14	N	28.00	2.47	27.22	23.92	11.29	11.13
20	N	25.23	-2.25	27.31	24.98	12.42	9.99
22	S	27.37	-0.81	26.34	25.43	11.45	9.98
25	N	25.99	-1.70	26.59	24.01	12.33	11.68
27	N	22.81	7.51	26.26	24.33	13.81	13.39
32	N	25.02	-0.14	26.87	23.39	10.94	13.97
34	S	28.54	2.59	25.55	23.24	11.49	12.11
36	N	23.67	-2.10	26.43	23.60	12.02	12.00
39	S	25.08	-0.06	28.20	23.37	11.35	10.27
41	S	24.66	1.29	22.06	22.71	10.98	13.66
43	P	32.02	5.76	22.62	22.62	11.00	14.15
47	P/I	34.39	10.14	19.87	21.12	12.85	13.02
48	P/I	37.34	12.32	18.14	20.09	12.20	13.18
50	I	44.44	19.70	15.17	18.01	12.17	12.80
53	P	26.68	-0.05	22.42	22.45	12.48	16.48
55	I	33.31	10.05	20.37	21.02	11.29	14.28
57	I	31.92	6.66	20.64	22.18	12.66	12.83
60	N	24.92	-0.91	27.00	22.91	14.05	11.34

[a] N = no degradation; S = selective utilization of n-alkanes C_{12} to C_{19}; P = partial removal n-alkane peaks still discernible; I = only isoprenoid peaks remaining.

[b] Corrected by subtracting respective uninoculated control values.

[c] The %'s of each oil fraction were calculated based on the initial weight of oil present in each flask and not as a percentage of the residual weight.

TABLE 14. GRAVIMETRIC AND G.C. CHANGES IN PRUDHOE BAY OIL BROUGHT ABOUT BY INCUBATION WITH WATER COLUMN SAMPLES FROM PT. ANGELES HARBOR (PEABODY CREEK, JUNE, 1979).

				% Composition of Residual Oil[c]			
Incubation Time(Days)	G.C.[a] Profile	% Weight Loss of Oil	Corrected[b] % Weight Loss	Saturates	Aromatics	Asphaltenes	NSO's
14	N	26.73	1.20	25.75	24.22	11.05	12.04
20	P	29.87	2.39	23.39	25.58	11.57	10.74
22	S	26.93	0.37	26.56	25.10	12.54	10.60
25	P	34.65	6.95	19.29	22.37	11.69	13.77
29	C	41.35	13.80	14.75	19.91	10.76	14.29
32	C	41.86	16.69	15.00	19.92	11.59	13.39
34	C	43.59	17.64	15.22	19.64	10.22	12.79
36	C	39.12	15.24	14.84	20.33	11.57	14.83
39	C	39.46	14.33	14.35	20.26	10.43	14.36
41	C	39.95	16.58	14.14	18.93	11.30	15.71
43	C	43.94	17.69	13.59	18.31	12.11	14.30
47	C	41.67	17.42	13.83	18.57	-[d]	15.41
48	C	41.04	16.01	14.31	18.30	12.38	15.27
50	C	43.01	18.28	13.53	18.04	12.84	14.73
53	C	43.17	16.44	13.49	17.75	12.32	14.76
55	C	43.52	20.26	13.75	18.19	12.05	14.80
57	C	46.06	20.81	13.31	17.55	10.78	13.07
60	C	45.97	20.80	13.48	17.87	12.40	12.08
62	C	44.64	16.07	13.05	17.36	12.41	12.50

[a] N = no degradation; S = selective utilization of n-alkanes C_{12} to C_{19}
P = partial removal; n-alkane peaks still discernible; C = complete utilization of n-alkanes and isoprenoids.

[b] Corrected by subtracting respective uninoculated control values

[c] The %'s of each oil fraction were calculated based on the initial weight of oil present in each flask and not as a percentage of the residual weight.

[d] fraction lost

TABLE 15. GRAVIMETRIC AND G.C. CHANGES IN PRUDHOE BAY OIL BROUGHT ABOUT BY INCUBATION WITH WATER COLUMN SAMPLES FROM DUNGENESS SPIT #2 (JUNE 1979).

Incubation Time (Days)	G.C.[a] Profile	% Weight Loss of Oil	Corrected[b] % Weight Loss	% Composition of Residual Oil[c]			
				Saturates	Aromatics	Asphaltenes	NSO's
14	N	29.41	3.88	28.15	24.89	10.97	9.14
20	S	26.42	-1.07	27.84	24.97	10.85	10.70
22	S	26.40	-0.16	28.64	24.91	11.68	9.31
25	N	25.81	-1.88	26.70	23.92	11.19	13.20
27	N	25.23	9.93	26.60	23.77	12.86	11.93
29	P	31.50	3.93	23.19	23.44	11.41	11.84
32	C	48.91	23.75	13.19	17.44	8.85	12.88
34	C	39.57	13.62	14.96	20.88	10.98	14.40
36	I	48.44	24.55	14.68	17.19	9.55	10.76
39	C	48.31	23.19	11.86	16.26	19.36	12.71
41	P	32.22	8.84	19.77	21.66	13.51	14.01
43	C	41.76	15.51	14.44	19.44	11.74	14.51
47	C	38.89	15.04	13.84	19.08	13.08	15.08
48	C	40.54	15.51	14.33	19.10	12.70	15.40
50	C	37.47	17.73	14.45	19.36	12.93	16.72
53	C	53.67	26.95	11.06	15.14	11.63	9.79
55	C	39.56	16.30	13.52	19.40	13.69	15.07
57	C	45.11	19.86	13.13	17.54	10.52	12.91
60	C	43.18	18.01	13.65	18.65	12.58	12.15
62	S	27.26	-1.35	24.84	22.46	13.53	10.46

[a] N = no degradation; S = selective utilization of n-alkanes C_{16} to C_{19}; P = partial removal, n-alkane peaks still discernible; I = only isoprenoid peaks remaining; C = complete utilization of n-alkanes and isoprenoids.

[b] corrected by subtracting respective uninoculated control values.

[c] The %'s of each oil fraction were calculated based on the initial weight of oil present in each flask and not as a percentage of the residual weight.

Radiometric Studies

The rates of release of $^{14}CO_2$ and length of lags from Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-anthracene or [9-^{14}C]-phenanthrene as a result of incubation of water column samples from northern Puget Sound, supplemented with nitrogen and phosphorus, are presented in Table 16. Data showing the length of lags and yields of $^{14}CO_2$ from the April 1979 sample series are illustrated in Figure 12. With one exception (August 1978 sample from Samish Island) the lag period before 5000 DPM of $^{14}CO_2$ is released is shorter for [1-^{14}C]-napthalene-"spiked" oil than for n-[1-^{14}C]-hexadecane-"spiked" oil, and the rate of release of $^{14}CO_2$ is always faster from [1-^{14}C]-naphthalene than from n-[1-^{14}C]-hexadecane. A comparison of the data obtained for the April 1979 samples (Table 16 and Figure 12), when [9-^{14}C]-anthracene and [9-^{14}C]-phenanthrene were included for comparative purposes, indicates that naphthalene is metabolized first, but on the basis of the evolution of $^{14}CO_2$ relatively incompletely. This is in contrast to the catabolism of [9-^{14}C]-phenanthrene, which requires a longer lag before $^{14}CO_2$ is detected, but yields very much higher levels of $^{14}CO_2$. The rate of utilization of these two aromatics is much faster than the metabolism of n-[1-^{14}C]-hexadecane or [1-^{14}C]-anthracene (Table 16). This latter aromatic is degraded to a greater extent by water column samples from E. Fidalgo than those from the "pristine" Pt. Partridge area. A comparison of the G.C. profiles of recovered oils (data not reported) indicates that extensive release of $^{14}CO_2$ has occurred from oil samples "spiked" with [1-^{14}C]-naphthalene and [9-^{14}C]-phenanthrene before changes are noted in the n-alkane and isoprenoid components of recovered oils. With the exception of [1-^{14}C]-naphthalene where there was very little difference in the pattern of $^{14}CO_2$ from water column samples from E. Fidalgo and Pt. Partridge shortest lags (i.e. time before $^{14}CO_2$ was rapidly released) were always observed for E. Fidalgo water column samples.

The rates of release of $^{14}CO_2$ and length of lags from a similar series of experiments involving water column samples from the Pt. Angeles area are presented in Table 17 and the length of lags and yields of $^{14}CO_2$ are presented in Figure 13. As previously noted with water column samples, $^{14}CO_2$ was released with the shortest lag time from [1-^{14}C]-naphthalene and, with the exception of [9-^{14}C]-phenanthrene, the fastest rates of release (Table 17) were obtained with naphthalene. The metabolism of phenanthrene by the microflora present in water from oil sites again yielded t[illegible] greatest amount of $^{14}CO_2$. Water column samples from Peabody Creek site were the most active in releasing $^{14}CO_2$ from all substrates tested with least difference being shown on [1-^{14}C]-naphthalene. Samples from this site also yielded higher levels of $^{14}CO_2$ from labelled hexadecane, anthracene and naphthalene "spiked" oil than water samples from Dungeness Spit and Ediz Hook. A comparison of the G.C. profiles of recovered oils again indicates that extensive metabolism of naphthalene and phenanthrene occurs before significant changes in the G.C. profiles of the saturate fraction are detectable. Yields of 20,000 DPM of $^{14}CO_2$ from the hexadecane experiments were noted to occur in this experiment without any major changes (e.g. N/S or S/P category) in the n-alkane profile

TABLE 16. RATE[a] of $^{14}CO_2$ EVOLUTION FROM n-[1-^{14}C]-HEXADECANE, [1-^{14}C]-NAPHTHALENE, [9-^{14}C]-ANTHRACENE AND [9-^{14}C]-PHENANTHRENE BY WATER COLUMN SAMPLES FROM THE NORTHERN PUGET SOUND AREA.

Date	Site	n-[1-^{14}C]-Hexadecane		[1-^{14}C]-Naphthalene		[9-^{14}C]-Anthracene		[9-^{14}C]-Phenanthrene	
		lag (days)	μg/L/day	lag (days)	μg/L/day	lag (days)	μg/L/day	lag (days)	μg/L/day
Aug. 1978	Samish Island	11.0	65.7	12.0	731	-[b]	-	-	-
	East Fidalgo	6.0	184	4.0	990	-	-	-	-
	Pt. Partridge	8.0	18.7	2.0	164	-	-	-	-
Nov. 1978	Samish Island	12.5	45.0	8.5	233	-	-	-	-
	East Fidalgo	9.0	97.0	3.5	240	-	-	-	-
	Pt. Partridge	13.5	22.3	9.8	140	-	-	-	-
April 1979	East Fidalgo	6.0	97.3	3.0	451	11.0	61.6	8.0	1083
	Pt. Partridge	9.0	65.7	2.5	1112	14.5	14.9	10.5	1083

[a] based on the rate of release of $^{14}CO_2$ from n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-anthracene and [9-^{14}C]-phenanthrene from "spiked" Prudhoe Bay oil.

[b] samples not examined

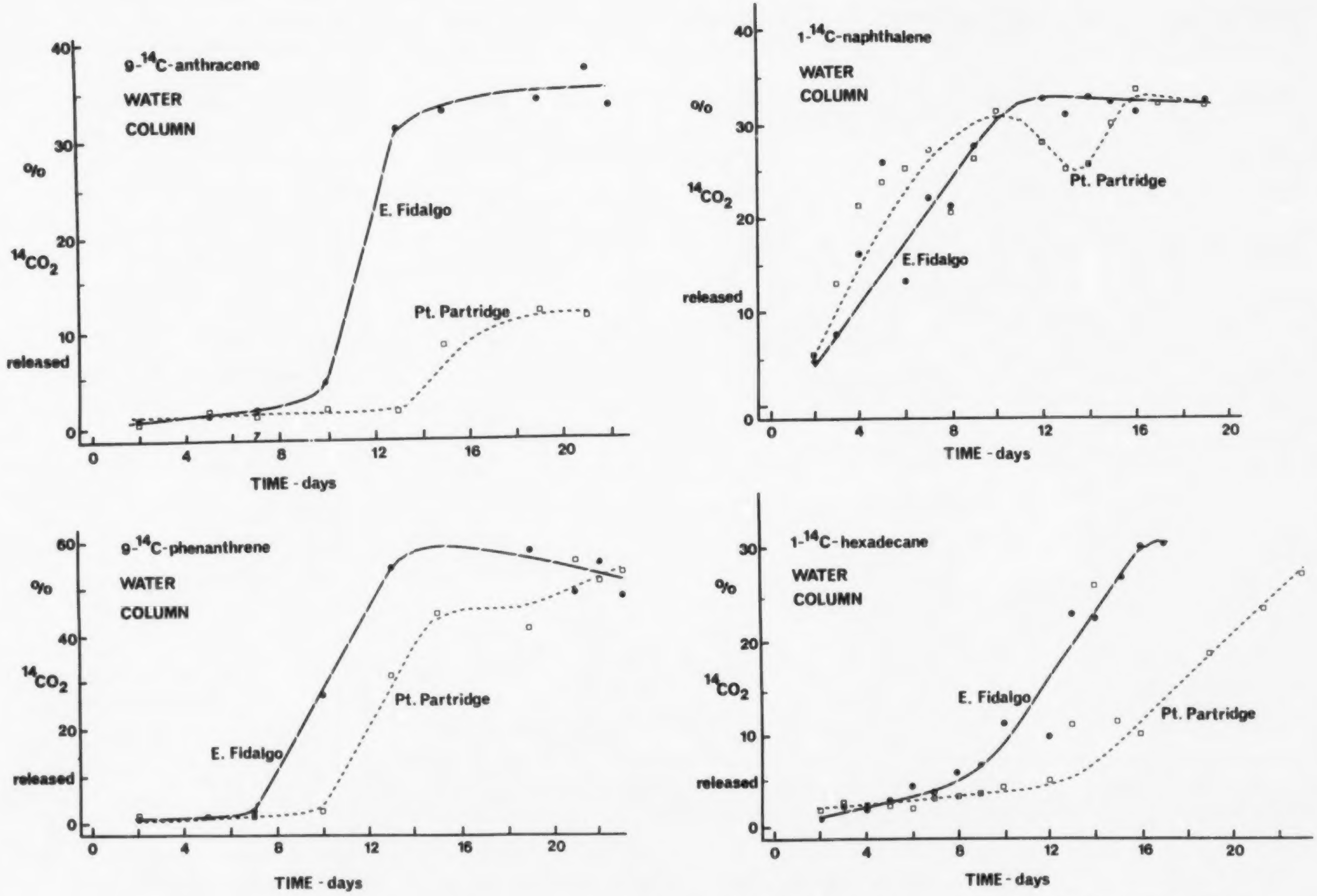

Figure 12. $^{14}CO_2$ evolution patterns for Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-phenanthrene or [9-^{14}C]-anthracene for water column samples from E. Fidalgo and Pt. Partridge (April, 1979).

TABLE 17. RATE[a] OF $^{14}CO_2$ EVOLUTION FROM n-[1-^{14}C]-HEXADECANE, [1-^{14}C]-NAPHTHALENE, [9-^{14}C]-ANTHRACENE AND [9-^{14}C]-PHENANTHRENE BY WATER COLUMN SAMPLES FROM THE PT. ANGELES AREA.

Date	Site	n-[1-^{14}C]-Hexadecane		[1-^{14}C]-Naphthalene		[9-^{14}C]-Anthracene		[9-^{14}C-Phenanthrene	
		lag (days)	μg/L/day	lag (days)	μg/L/day	lag(days)	μg/L/day	lag (days)	μg/L/day
Oct. 1978	Freshwater Bay	8.0	65.9	6.0	598	-[b]	-	-	-
	Pt. Angeles- Ediz Hook #1	10.0	138	6.0	472	-	-	-	-
	Pt. Angeles- Southside (Peabody Creek)	6.0	39.6	4.0	428	-	-	-	-
	Dungeness Spit #2	7.0	78.3	7.0	236	-	-	-	-
Jan. 1979	Freshwater Bay	12.0	113	4.0	545	-	-	-	-
	Pt. Angeles- Southside (Peabody Creek)	8.5	139	4.0	723	-	-	-	-
	Dungeness Spit #2	12.5	67	4.0	535	-	-	-	-
June 1979	Pt. Angeles- Ediz Hook #1	8.5	34	6.0	464	14.0	21.8	11.5	2750
	Pt. Angeles- Southside (Peabody Creek)	6.0	133	2.0	654	7.5	102	5.5	1640
	Dungeness Spit #2	10.0	42.4	4.0	308	10.5	47.0	9.5	2090

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[a] based on the rate of release of $^{14}CO_2$ from n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-anthracene and [9-^{14}C]-phenanthrene from "spiked" Prudhoe Bay oil.

[b] samples not examined

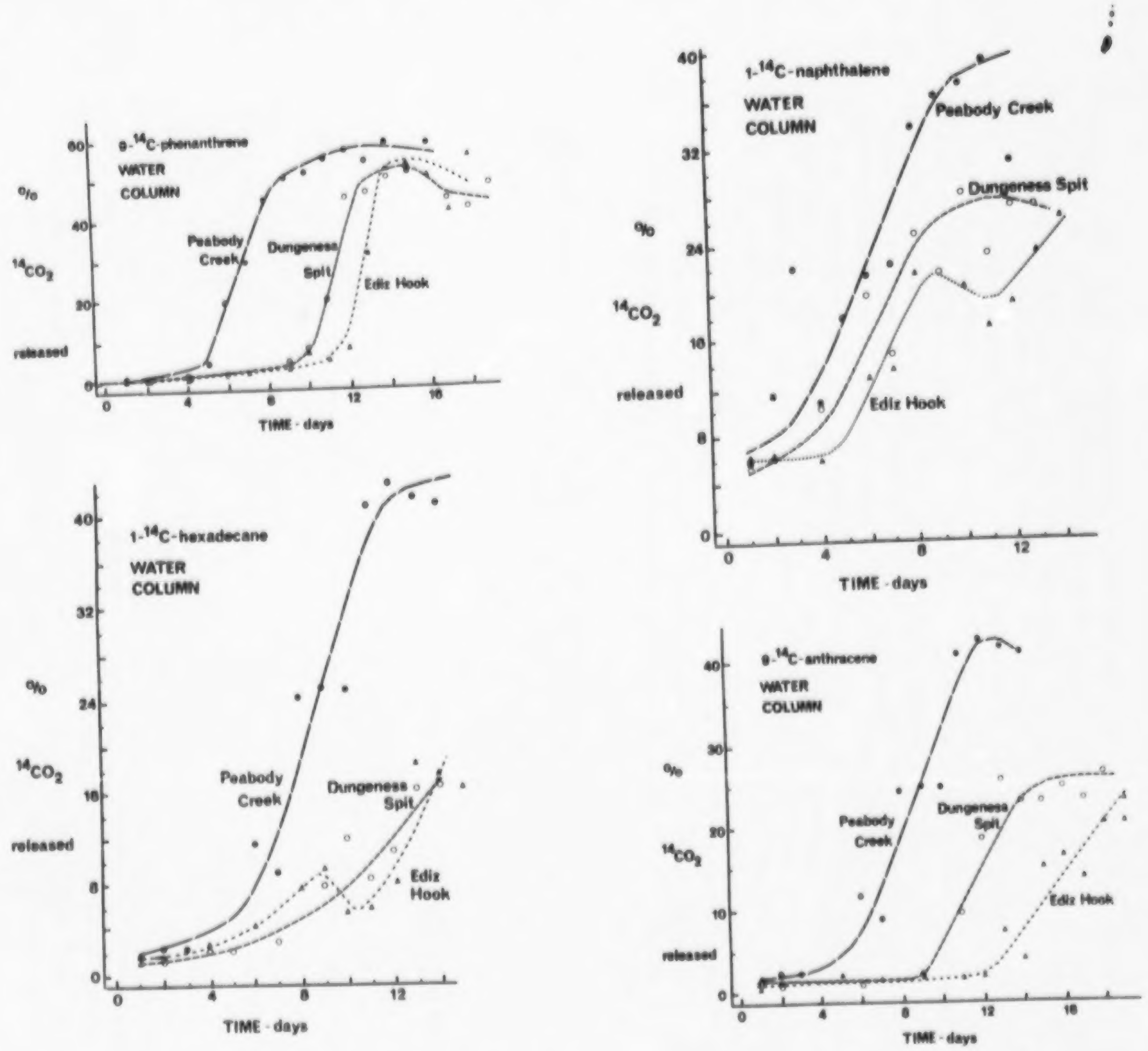

Figure 13. $^{14}CO_2$ evolution patterns for Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-phenanthrene or [9-^{14}C]-anthracene for water column samples from Ediz Hook, Peabody Creek and Dungeness Spit #2 (June, 1979).

being detectable by the G.C. procedure used.

Data on the rate of release of $^{14}CO_2$ and length of lags from a series of experiments using sub-tidal sediments are presented in Table 18 and the June 1979 data showing length of lags and yields of $^{14}CO_2$ are presented in Figure 14. On the basis of length of lag time,[1-^{14}C]-naphthalene again is the first hydrocarbon of those studied to yield significant levels of $^{14}CO_2$. The fastest rates of $^{14}CO_2$ evolution occur with [1-^{14}C]-naphthalene- and [9-^{14}C]-phenanthrene-"spiked" oils (Table 18). Sub-tidal samples from Ediz Hook and Peabody Creek were more active, i.e. shorter lag before rapid rate of $^{14}CO_2$ evolution is observed, than Dungeness Spit sediment in releasing $^{14}CO_2$ from [1-^{14}C]-hexadecane, [9-^{14}C]-phenanthrene and [9-^{14}C]-anthracene. In contrast the patterns of $^{14}CO_2$ evolution from [1-^{14}C]-naphthalene are similar for all three sub-tidal sediment samples. Since very little change was detectable in the n-alkane and isoprenoid profiles of the saturate fractions as determined by G.C., the metabolism of these aromatics is taking place before n-alkane utilization. The release of $^{14}CO_2$ from [9-^{14}C]-phenanthrene (Figure 14) is in the form of a biphasic curve for the sub-tidal sediment samples from Pt. Angeles harbor. The sub-tidal sediment samples from Ediz Hook (Pt. Angeles harbor) also yielded a biphasic curve for [9-^{14}C]-anthracene catabolism.

Data on the production of $^{14}CO_2$ in a similar series of experiments using beach samples as sources of inoculum are presented in Table 19. The results are similar to those previously reported regarding the preferential utilization of naphthalene before hexadecane.

Utilization of Other Oils Imported into the Puget Sound Area

In 1978 the oil refineries operating at Anacortes, Washington were importing oil from Sumatra (Minas), the Middle East - Jabal Dhanna (Murban) and Malaysia (Seria). Minas is physically a different oil as it is a waxy crude with a pour point of approximately 95°F (i.e. it is a solid at room temperature) whereas Murban and Seria are liquids at room temperature. The chemical compositions of these oils, as well as Prudhoe Bay for comparative purposes, are presented in Table 20 and the G.C. profile of the saturate fractions in Figures 3, 4, 5 and 6. The results of an experiment to determine the biodegradability of these new oils using oil-degrading marine cultures enriched on Prudhoe Bay oil are presented in Table 21. Murban and Seria, like Prudhoe Bay, were readily used by these cultures at both 8°C and 20°C. Very little activity, as judged by changes in the G.C. profile of the saturate fraction, was observed when Minas, the "solid" oil from Sumatra, was used as sole carbon source even when supplemented with nitrogen and phosphorus.

Data on the utilization of these new oils by microorganisms present in water column and beach samples from the northern Puget Sound and Pt. Angeles area are presented in Table 22. Changes in the G.C. profiles of the saturate fraction of Murban and Seria were readily brought about by samples from areas likely to have been contaminated with petroleum and/or its products (i.e.

TABLE 18. RATE[a] OF $^{14}CO_2$ EVOLUTION FROM n-[1-^{14}C]-HEXADECANE, [1-^{14}C]-NAPHTHALENE, [9-^{14}C]-ANTHRACENE AND [9-^{14}C]-PHENANTHRENE BY SUB-TIDAL SEDIMENT SAMPLES FROM THE PT. ANGELES AREA.

		n-[1-^{14}C]-Hexadecane		[1-^{14}C]-Naphthalene		[9-^{14}C]-Anthracene		[9-^{14}C]-Phenanthrene	
Date	Site	lag (days)	μg/Kg[b]/day	lag (days)	μg/Kg[b]/day	lag (days)	μg/Kg[b]/day	lag (days)	μg/Kg[b]/day
Oct. 1979[c]	Freshwater Bay	14.0	1175	9.0	1121	-[d]	-	-	-
	Pt. Angeles-Ediz Hook #1	16.0	575	6.0	1799	-	-	-	-
	Pt. Angeles-Southside (Peabody Creek)	13.0	416	9.0	3057	-	-	-	-
June 1979[e]	Pt. Angeles-Ediz Hook #1	6.5	688	5.0	1756	6.5	235	6.5	17,930
	Pt. Angeles-Southside (Peabody Creek)	8.5	473	6.0	1660	9.0	1036	6.5	13,170
	Dungeness Spit #2	10.5	214	5.0	1215	13.0	291	8.5	7,999

---

[a] based on the rate of release of $^{14}CO_2$ from n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-anthracene and [9-^{14}C]-phenanthrene from "spiked" Prudhoe Bay oil.

[b] Kg (dry weight)

[c] degradative capability after 28 days incubation at 8°C = S, C and C for Freshwater Bay, Pt. Angeles, Ediz Hook #1 and Southside (Peabody Creek) respectively

[d] samples not examined

[e] degradative capability after 28 days incubation at 8°C = C, C and S for Pt. Angeles, Ediz Hook #1, Southside (Peabody Creek) and Dungeness Spit #2 respectively.

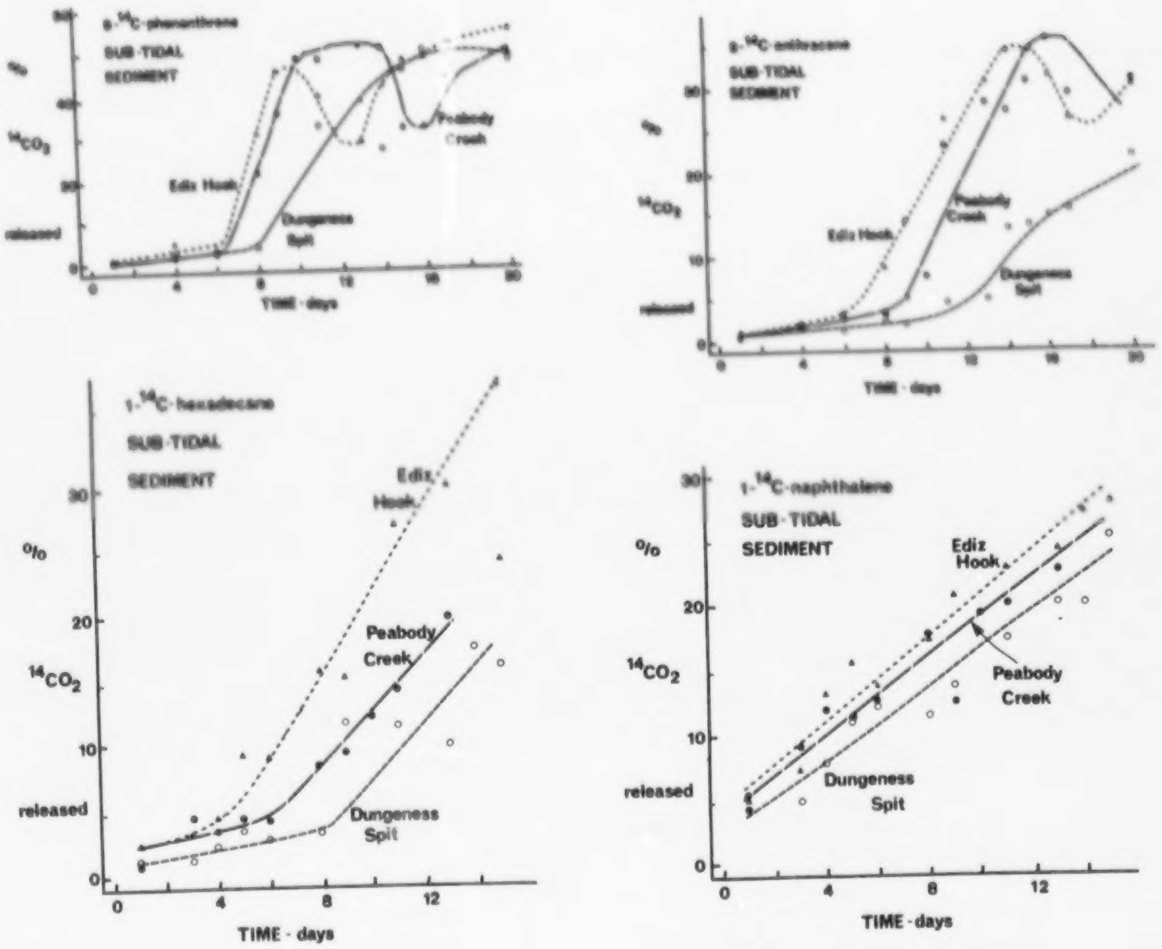

Figure 14. $^{14}CO_2$ evolution patterns for Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-phenanthrene or [9-^{14}C]-anthracene for sub-tidal sediments from Ediz Hook, Peabody Creek and Dungeness Spit #2 (June, 1979).

TABLE 19. RATE[a] of $^{14}CO_2$ EVOLUTION FROM n-[1-^{14}C]-HEXADECANE, [1-^{14}C]-NAPHTHALENE BY BEACH SAMPLES FROM THE PT. ANGELES AREA.

Date	Site	n-[1-^{14}C]-Hexadecane		[1-^{14}C]-Naphthalene	
		lag (days)	μg/kg[b]/day	lag (days)	μg/kg[b]/day
Jan. 1979	Freshwater Bay	10.8	131	2	1737
	Pt. Angeles-Southside (Peabody Creek)	6.8	249	5.2	1032
	Dungeness Spit #2	18	22	8.6	1009

[a] based on the rate of release of $^{14}CO_2$ from n-[1-^{14}C]-hexadecane and [1-^{14}C]-naphthalene from "spiked" Prudhoe Bay oil.

[b] Kg (dry weight)

TABLE 20. CHEMICAL COMPOSITION OF OILS.

Crude Oil Fraction	% Composition of Oil[a]			
	Minas	Murban	Prudhoe Bay	Seria
Asphaltenes	18.8(±1.2)	5.2(±0.5)	7.3(±0.7)	2.1(±0.3)
Saturates	60.3(±4.5)	54.0(±1.0)	38.7(±0.7)	60.4(±0.4)
Aromatics	15.6(±0.1)	34.8(±6.6)	36.4(±0.5)	33.8(±1.3)
NSO's	7.9(±1.9)	8.6(±3.7)	18.7(±2.3)	9.5(±1.4)

[a] average weight percent composition (±1 standard deviation) of three replicate samples after "topping" for 18 hrs at 30°C under forced draft conditions.

TABLE 21. UTILIZATION[a] OF MINAS (SUMATRA) MURBAN (JABAL DHANNA) AND SERIA (MALAYSIA) BY OIL-DEGRADING ENRICHMENTS FROM NORTHERN PUGET SOUND.

		G.C. Status of Saturate Fraction			
		Water Column[b] (March Pt. Rd)		Intertidal Sediment[b] West Beach-Deception St. Park	
Oil	Incubation Time (days)	8°C	20°C	8°C	20°C
Minas	10	NS	-	NS	-
	15	-	NS	-	NS
	20	NS	-	NS	+/-
	30	-	NS	-	NS
Murban	10	NS	+	NS	+
	15	+/-	NS	+	NS
	20	NS	+	NS	+
	30	+	NS	+	NS
Seria	10	NS	+/-	NS	+
	15	+/-	NS	+	NS
	20	NS	+	NS	+
	30	+	NS	NS	NS

[a] G.C. pattern of the saturate profile, see Figures 4, 5 and 6 (- = no degradation; +/- = selective degradation; + = degraded profile).

[b] cultures (28 days at 8°C with added nitrogen and phosphorus).

[c] NS - no sample

TABLE 22. UTILIZATION OF MINAS, MURBAN AND SERIA OILS BY WATER AND BEACH SAMPLES - NORTHERN PUGET SOUND AND PT. ANGELES AREA (JUNE, 1979).

Sample	G.C. Status of Saturate Fraction		
	Minas	Murban	Seria
Northern Puget Sound East Fidalgo (water)	-[a]	+	+
Pt. Partridge (water)	-	-	-
Pt. Angeles Area Ediz Hook (water)	SL[b]	-	+
(beach)	-	-	+/-
Pt. Angeles Harbor Peabody Creek (water)	-	+	+
(beach)	+	+	SL
Dungeness Spit #2 (water)	-	SL	+
(beach)	+	-	SL

[a] G.C. status of saturate profile, see Figures 4, 5 and 6; (- = no degradation; +/- = selective degradation; + = degraded profile) after 28 days incubation at 8°C with added nitrogen and phosphorus.

[b] sample lost

E. Fidalgo and Pt. Angeles harbor-Peabody Creek). More water column samples contained microorganisms which were able to bring about chemical changes in Seria than Murban oil. Minas, the oil which is a solid at room temperature, was not susceptible to alteration by incubation with water column samples. However the n-alkanes present in this oil were readily metabolized by microorganisms present in beach samples. Sub-tidal samples from Pt. Angeles harbor-Ediz Hook and Peabody Creek as well as from Dungeness Spit (#2) also contained microbes which readily metabolized the n-alkanes in Minas oil.

Very little biodegradative activity was detectable with samples that did not receive a nutrient supplement of nitrogen and phosphorus.

Studies on the Biodegradation Process

Incubation Time

Data on the effect of extended incubation time at 8°C on the loss in weight and changes in the G.C. status of Prudhoe Bay oil recovered from incubations with water column samples are presented in Table 23. Of the two samples taken from northern Puget Sound, water from E. Fidalgo brought about changes in oil more quickly and completely if nitrogen and phosphorus were present than water from Pt. Partridge. In the absence of added nutrients no significant changes in the oil were detected over the 3 month incubation period.

The results from a similar study (Table 23) using water samples from the Pt. Angeles area indicate that, in the presence of added nitrogen and phosphorus, water from Peabody Creek was the most active in bringing about changes in Prudhoe Bay oil. Water samples from Dungeness spit also brought about major changes in oil but required a longer incubation time to initiate and complete the changes. The water samples from Ediz Hook produced variable changes in the parameters measured. In the absence of added nitrogen and phosphorus the weight of oil lost varied markedly whereas the status of the G.C. profiles was consistent and indicated that the n-alkanes and isoprenoids had not been utilized.

Utilization of Aromatics and Saturates

A qualitative glass capillary G.C. analysis of the aromatic fraction of Prudhoe Bay oil is shown in Figure 15. Peaks have been identified on the basis of retention time comparisons with authentic standards, or by comparison with similar chromatograms (25). Chrysene was added as a recovery standard and hexamethylbenzene (hmb) as an internal standard. The data in Figure 16 represent the changes as resolved by this technique in the aromatic components of Prudhoe Bay oil after 6, 10 and 14 days incubation with water column samples from E. Fidalgo supplemented with nitrogen and phosphorus. Changes brought about under similar cultural conditions with control (sterile) water samples are presented in Figure 17. The recovery of the aromatic components after 27 days incubation for a control, and for water column samples with and without a nutrient supplement of nitrogen and phosphorus are

TABLE 23. EFFECT OF INCUBATION TIME AT (8°C) ON THE UTILIZATION OF PRUDHOE BAY OIL BY WATER COLUMN SAMPLES FROM NORTHERN PUGET SOUND (APRIL 2-3, 1979) AND PT. ANGELES AREA (JUNE 18-20, 1979).

Site	Nitrogen Phosphorus Addition	Incubation Period-8°C (Months)	% Weight Loss		G.C. Profile[b]
			Total	Biodegradation[a]	
Northern Puget Sound					
East Fidalgo	-	1	27.53	-0.45	N
	-	2	27.08	0	N
	-	3	26.98	-0.10	N
	+	1	29.16	2.08	S
	+	2	43.97	16.89	C
	+	3	44.29	17.21	C
Pt. Partridge	-	1	28.45	1.37	N
	-	2	26.86	-0.24	N
	-	3	-[c]	-	-
	+	1	27.54	0.46	N
	+	2	26.21	-0.87	N
	+	3	48.98	21.90	I

TABLE 23. continued .....

	Nitrogen Phosphorus Addition	Incubation Period-8°C (Months)	% Weight Loss		G.C. Profile[b]
			Total	Biodegradation[a]	
Port Angeles Area					
Ediz Hook (Pilot Station)	-	1	26.52	-0.96	N
	-	2	-	-	-
	-	3	24.10	-2.98	N
	+	1	22.81	-4.97	N
	+	2	31.92	4.84	P
	+	3	23.71	-3.37	N
Peabody Creek	-	1	36.89	9.81	N
	-	2	26.87	-0.21	N
	-	3	20.15	-6.93	N
	+	1	41.39	14.31	C
	+	2	45.97	18.89	C
	+	3	-	-	-
Dungeness Spit #2	-	1	25.65	-1.43	N
	-	2	28.23	1.15	N
	-	3	25.02	-2.06	N
	+	1	31.50	4.42	P
	+	2	43.18	16.10	C
	+	3	43.69	16.61	C

[a] value of 27.08 (weight %) used a loss due to physical-chemical processes

[b] see Figure 3

[c] flask broken

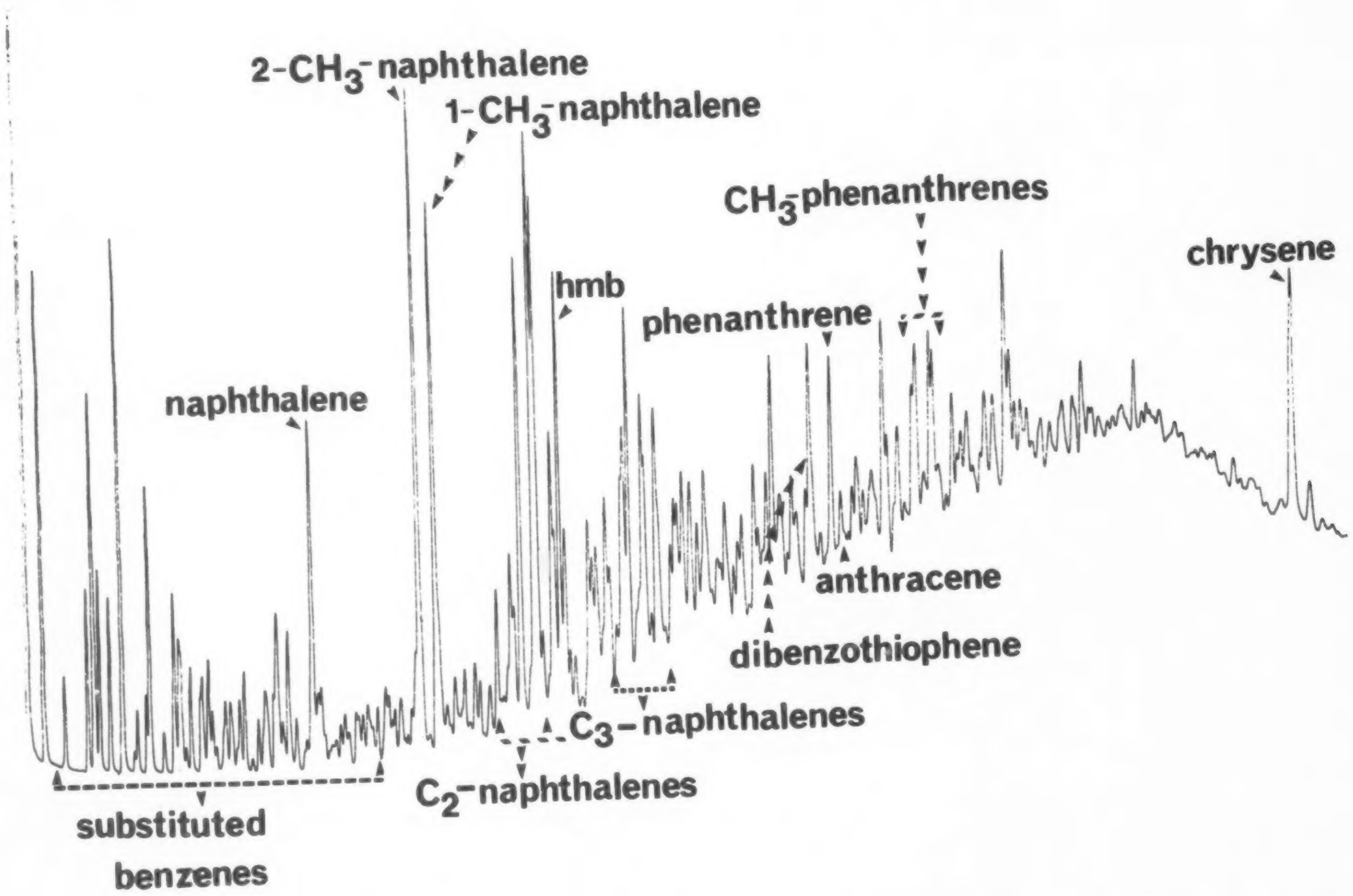

Figure 15. Typical G.C. profile (glass capillary column) of aromatic fraction of Prudhoe Bay oil (hmb = hexamethylbenzene).

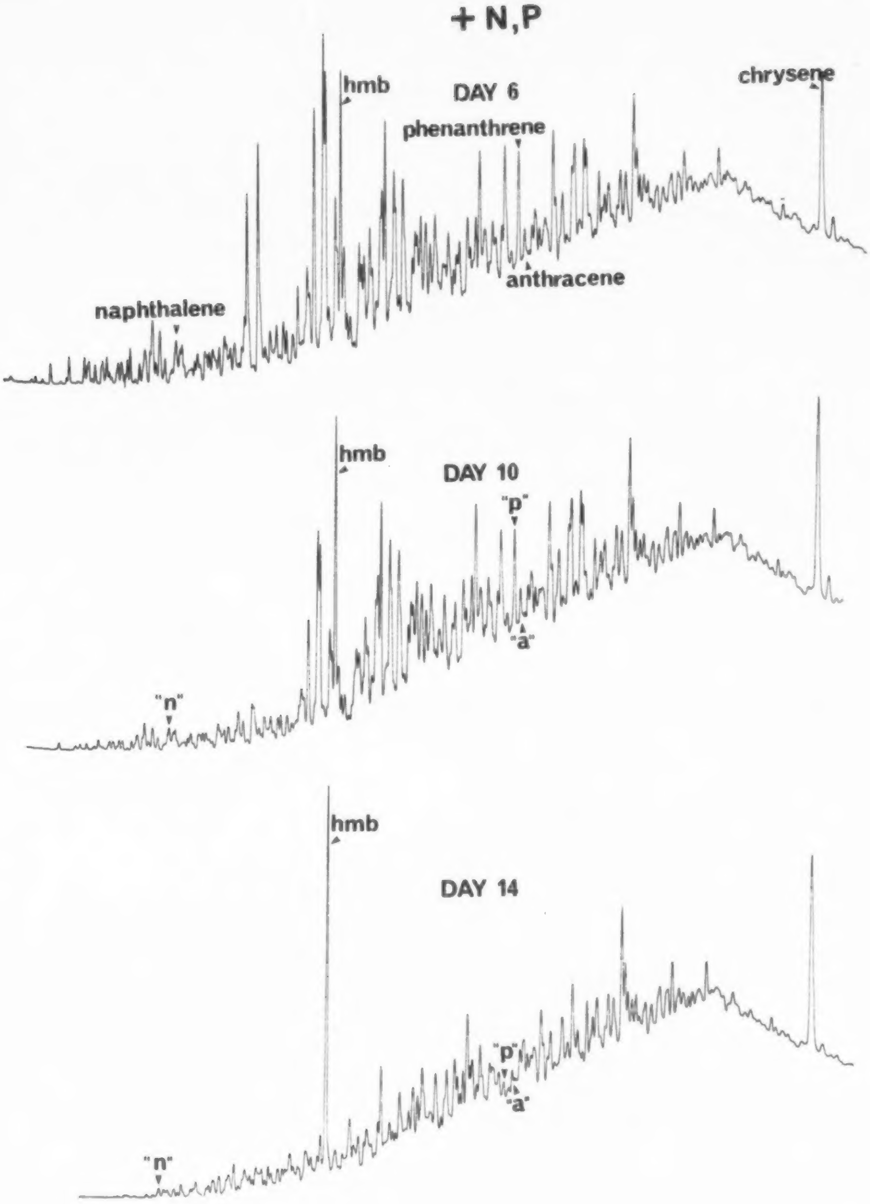

Figure 16. Changes in the G.C. profiles (glass capillary column) of the aromatic fraction of Prudhoe Bay oil after 6, 10 and 14 days incubation with water column samples from E. Fidalgo supplemented with nitrogen and phosphorus (+N,P) (hmb = hexamethylbenzene).

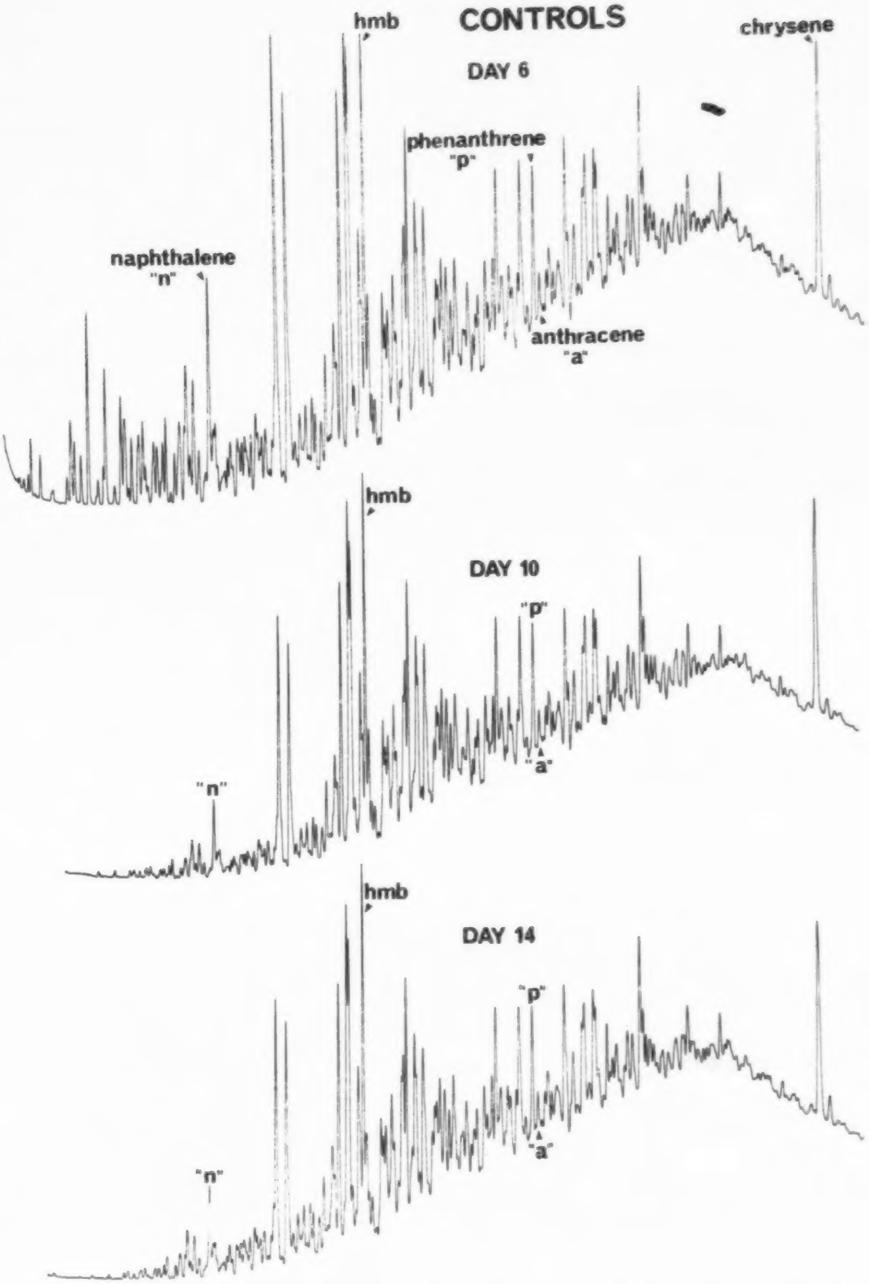

Figure 17. Changes in the G.C. profiles (glass capillary column) of the aromatic fraction of Prudhoe Bay oil recovered from control incubations (i.e. sterile artificial sea water) after 6, 10 and 14 days incubation (hmb = hexamethylbenzene).

presented in Figure 18.

The chromatograms from 3 day incubations (not included in this report) showed only a slight loss in naphthalene as a result of microbial activity when nitrogen and phosphorus were present. The G.C. (aromatic) profile of the 3 day control culture was similar to that of the 6 day sample which is presented in Figure 17. A comparison of the 6 day G.C. profiles (Figures 16 and 17) indicates that the more volatile components, e.g. substituted benzenes, naphthalene and 1-methyl-and 2-methyl-naphthalenes were being utilized during this period provided nitrogen and phosphorus had been added to the water. After 10 days incubation these compounds had been mostly used and the degradation of the dimethylnaphthalenes, dibenzothiophene and phenanthrene components of this oil had been initiated. The G.C. profile after 14 days incubation when the water column sample had been supplemented with nitrogen and phosphorus showed extensive utilization of aromatics up to and including the methylphenanthrenes. The chromatogram from 18 day incubations (not included in this report) differed little from those presented in Figures 16 and 17 for the 14 days sampling.

The samples recovered after 27 days incubation (Figure 18) indicate the extensive utilization of all of the aromatics resolved by this capillary technique. Considerable changes are also evident in the oil recovered from the culture which did not receive an addition of nitrogen and phosphorus. The lower molecular weight components, e.g. substituted benzenes, naphthalene, mono-methyl and dimethyl-benzenes, naphthalene derivatives, dibenzothiophene and phenanthrene show varying degrees of utilization as reflected by this G.C. technique.

Changes in the aromatic and saturate fractions of Prudhoe Bay oil after incubation for 27 days with water column samples from Peabody Creek, Pt. Partridge and E. Fidalgo with and without supplementation with nitrogen and phosphorus, are presented in Figures 19, 20, 21 and 22. Extensive utilization of the aromatic components occurred when water samples were supplemented with nitrogen and phosphorus (Figure 19). The microorganisms present in the Pt. Partridge water samples did not bring about as great a change in the aromatic G.C. profile as did those from E. Fidalgo and Peabody Creek. The "control" aromatic profile for a 27 day incubation for comparative purposes is presented in Figure 20. Marked changes in these aromatic components also were observed even when nitrogen and phosphorus were not added to incubation mixtures (Figure 20). However these changes were not as great as when the nutrients had been added. The microbes present in the water samples from Peabody Creek and Pt. Partridge brought about greater changes in the aromatic fraction than those present in the E. Fidalgo water sample in the absence of added nutrients.

The changes in the saturate content of recovered oil, under nitrogen and phosphorus-enriched conditions and in the absence of added nutrient supplements, are presented in Figures 21 and 22 respectively. The n-alkanes and isoprenoids were completely used when nitrogen and phosphorus had been

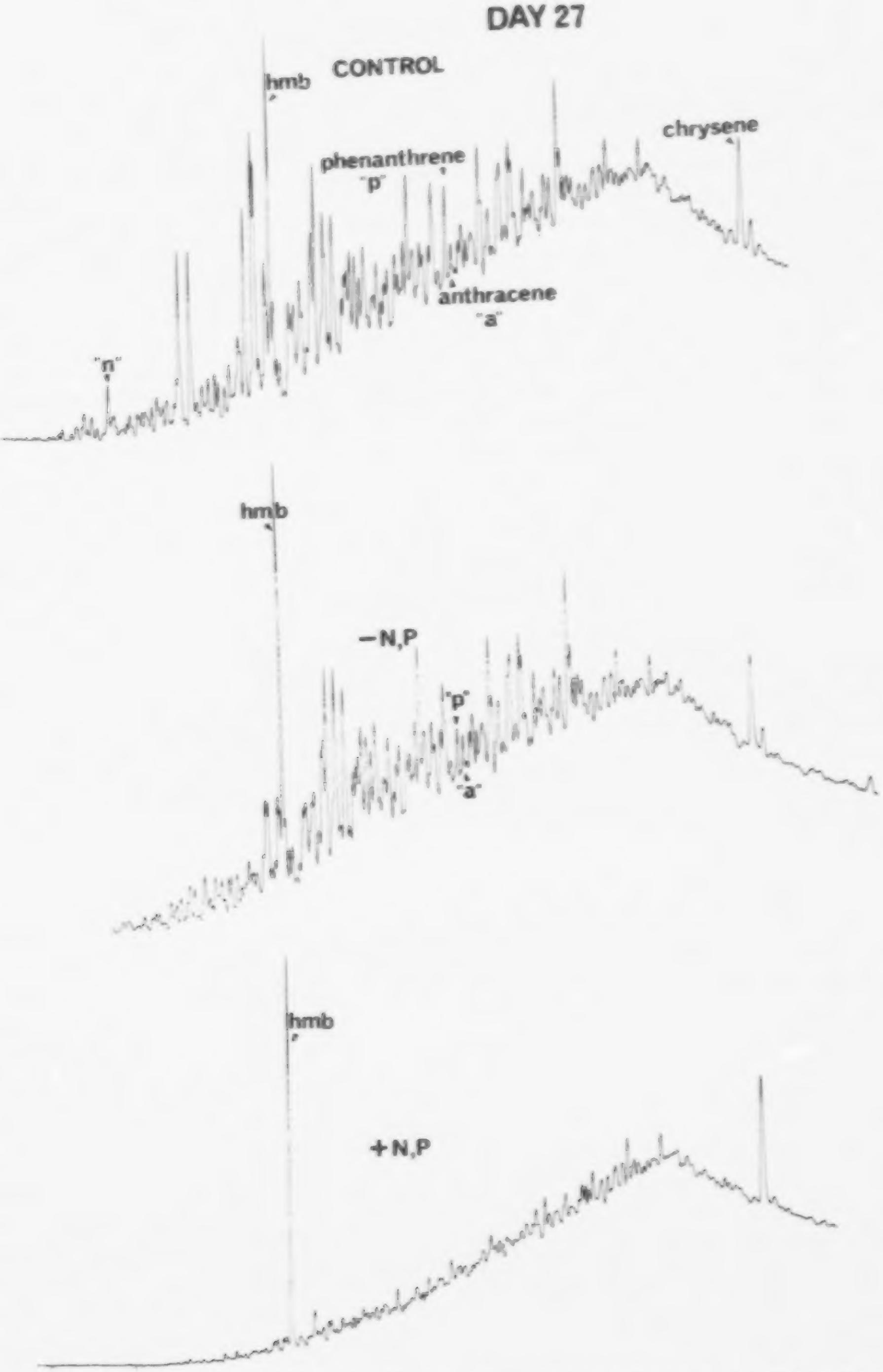

Figure 18. Changes in the G.C. profiles (glass capillary column) of the aromatic fraction of Prudhoe Bay oil recovered after 27 days incubation; control = sterile artificial sea water and E. Fidalgo water column sample without (-N,P) and with (+N,P) addition of nitrogen and phosphorus (hmb = hexamethylbenzene).

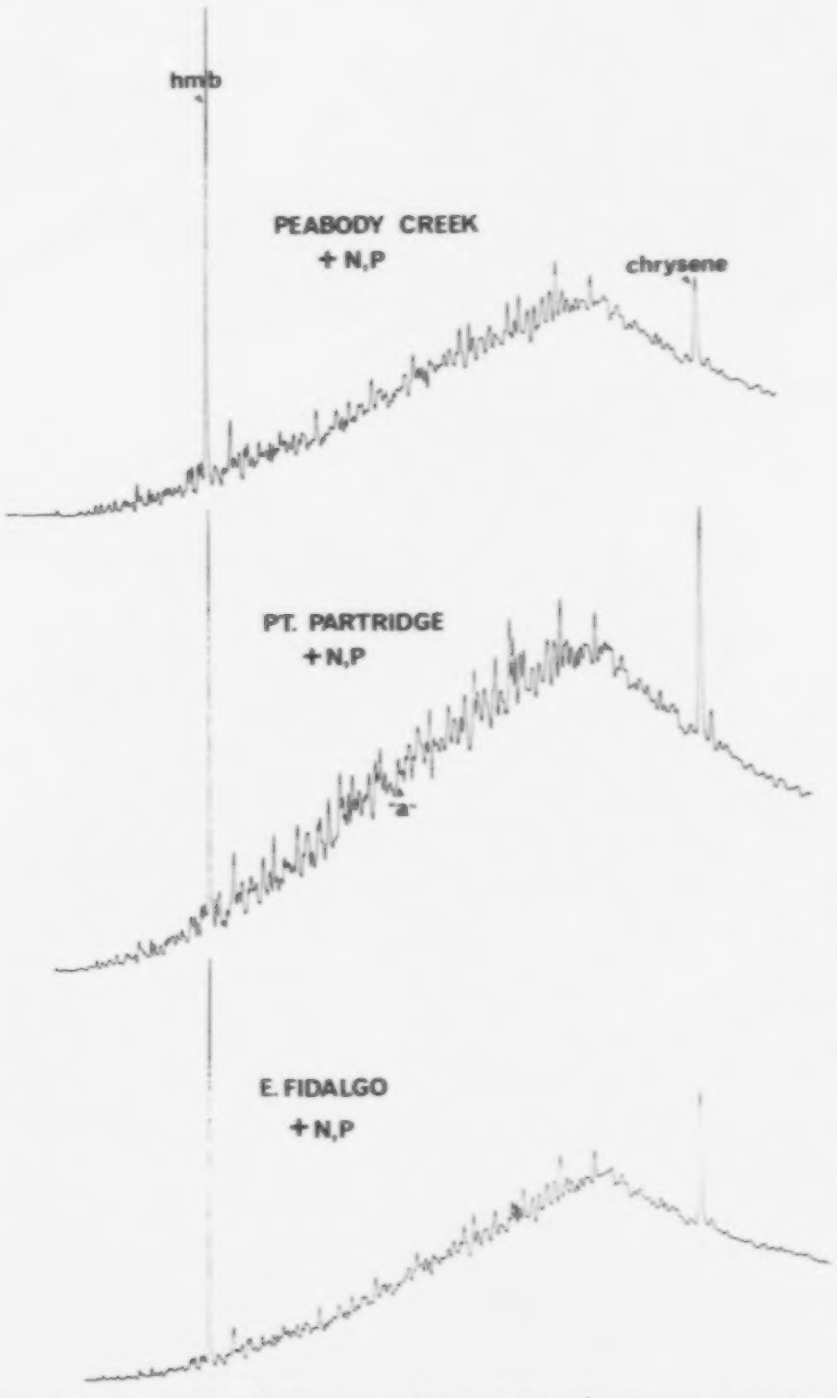

Figure 19. G.C. profiles (glass capillary column) of aromatic fraction of Prudhoe Bay oil recovered after 27 days incubation with water from one of Peabody Creek, Pt. Partridge or E. Fidalgo supplemented with nitrogen and phosphorus (+N,P) (hmb = hexamethylbenzene).

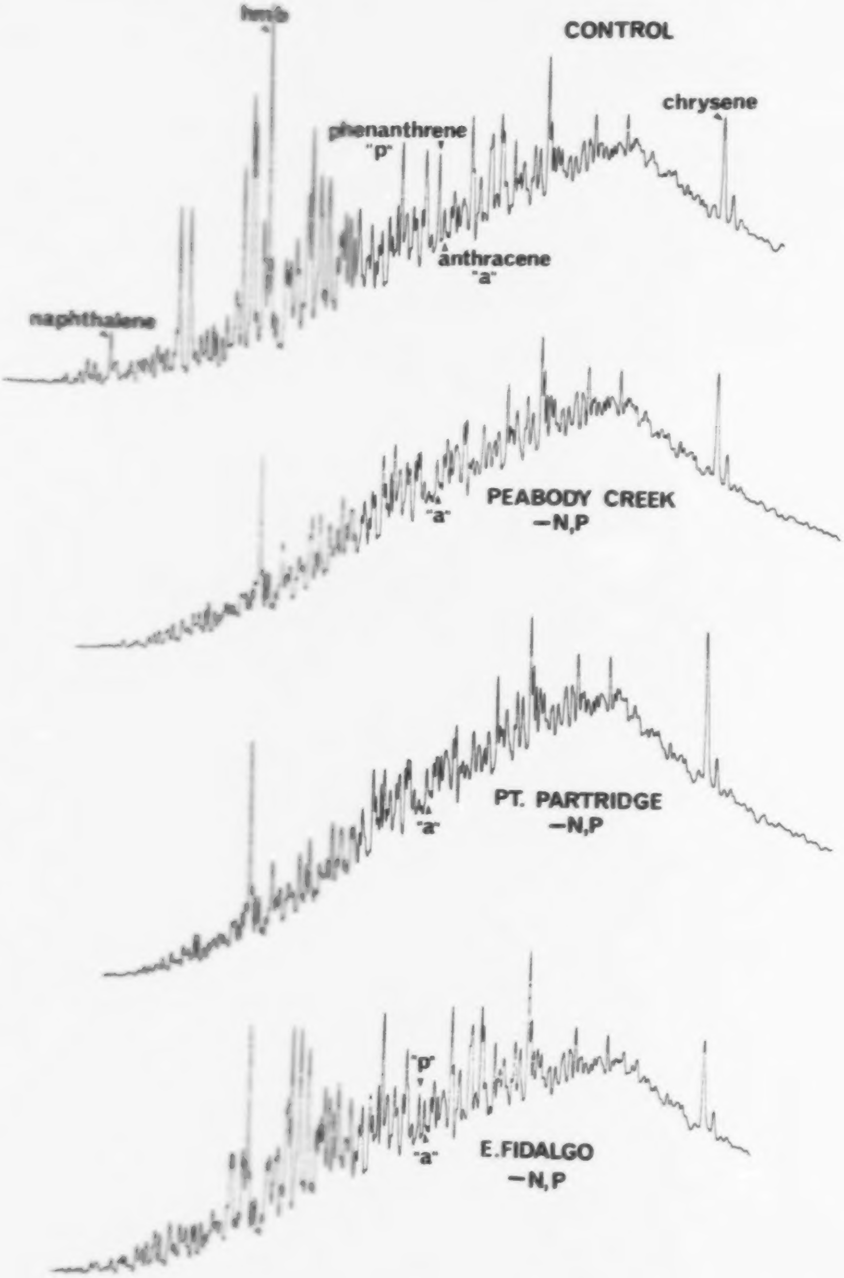

Figure 20. G.C. profiles (glass capillary column) of aromatic fraction of Prudhoe Bay oil recovered after 27 days incubation from a control (i.e. sterile artificial sea water) and water from one of Peabody Creek, Pt. Partridge or E. Fidalgo without nutrient supplementation (-N,P) (hmb = hexamethylbenzene).

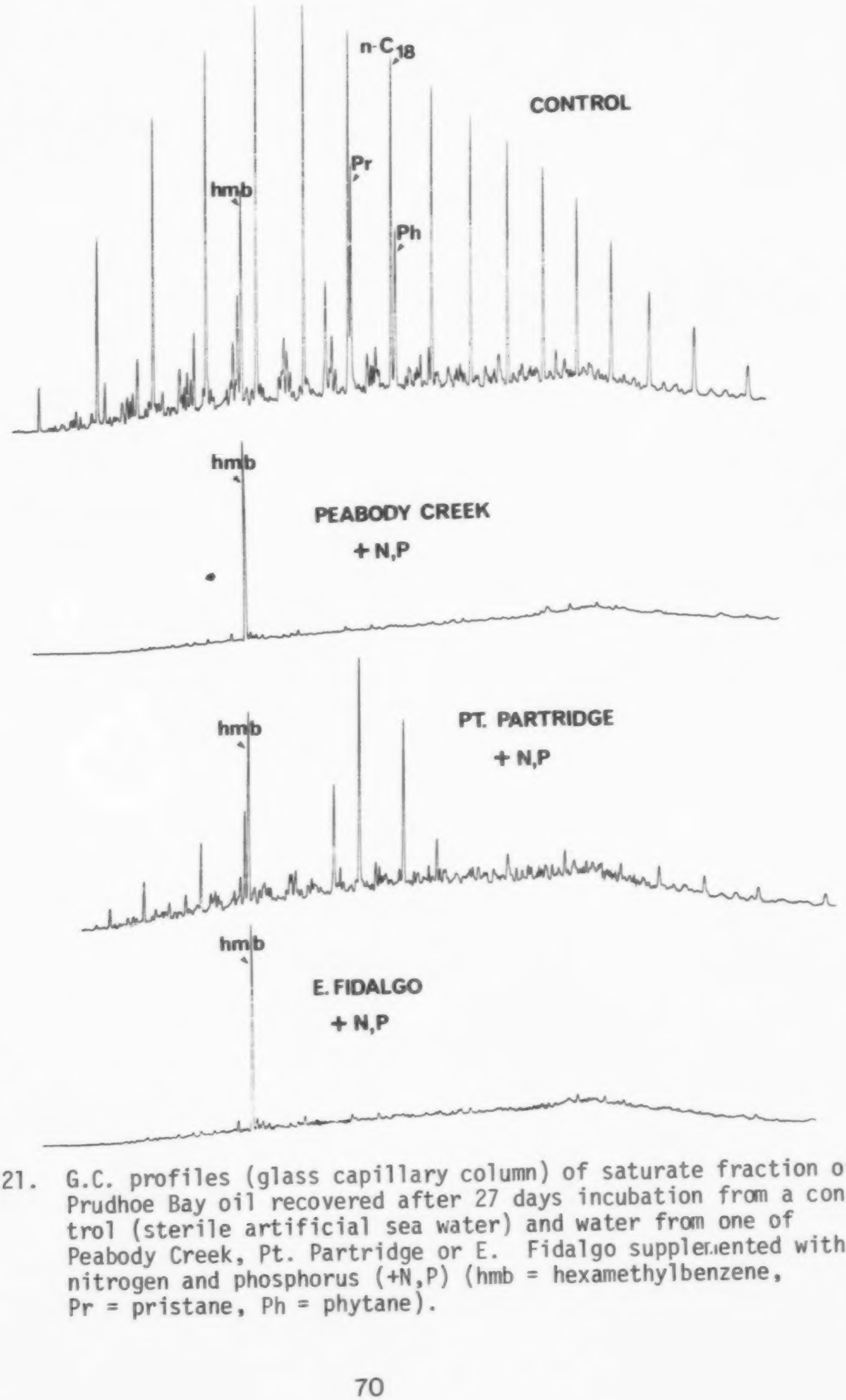

Figure 21. G.C. profiles (glass capillary column) of saturate fraction of Prudhoe Bay oil recovered after 27 days incubation from a control (sterile artificial sea water) and water from one of Peabody Creek, Pt. Partridge or E. Fidalgo supplemented with nitrogen and phosphorus (+N,P) (hmb = hexamethylbenzene, Pr = pristane, Ph = phytane).

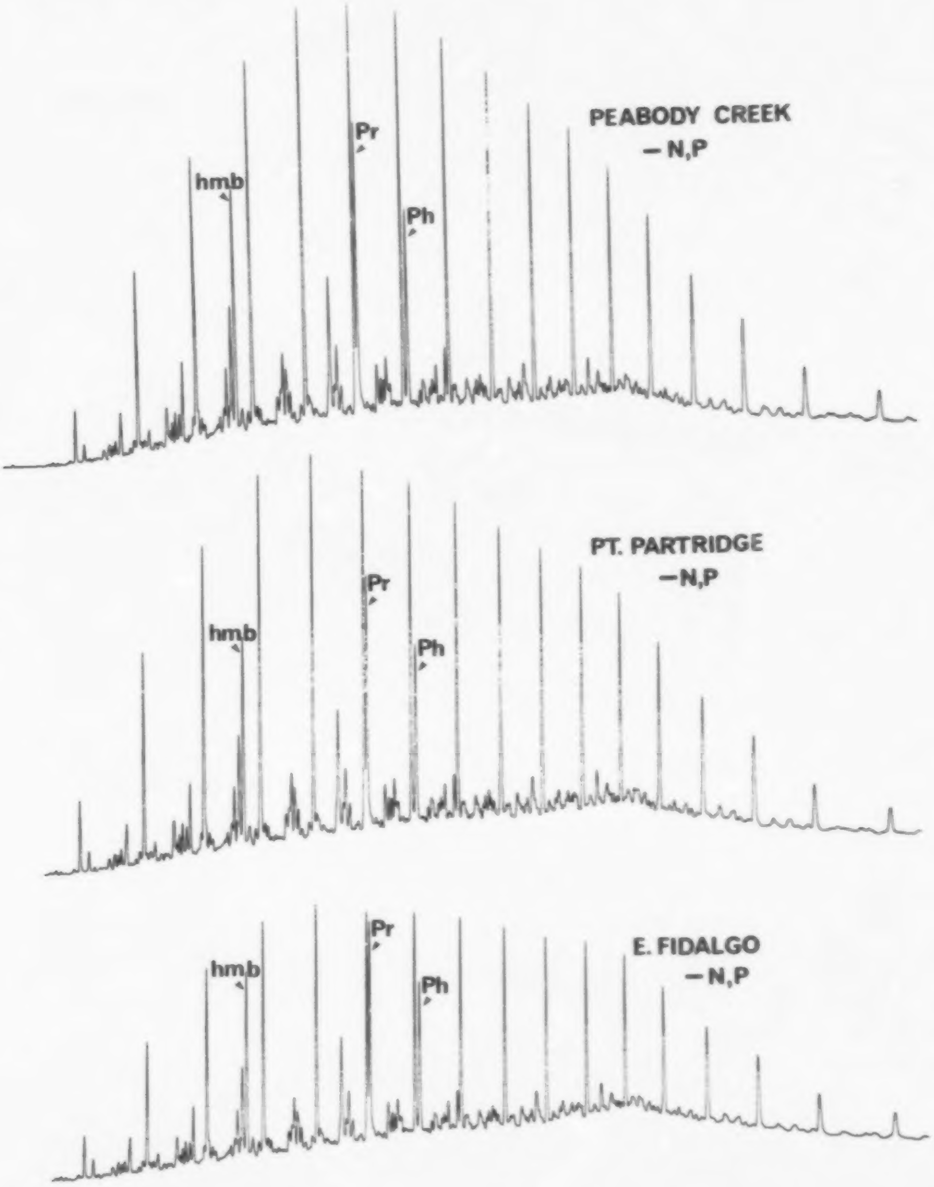

Figure 22. G.C. profiles (glass capillary column) of saturate fraction of Prudhoe Bay oil recovered after 27 days incubation with water from one of Peabody Creek, Pt. Partridge or E. Fidalgo without nutrient supplementation (-N,P) (hmb = hexamethylbenzene, Pr = pristane, Ph = phytane).

added to water column samples from Peabody Creek and E. Fidalgo. Residual peaks (possibly isoprenoids) are still clearly visible in the G.C. profile of the saturates in the oil recovered from the Pt. Partridge incubation. In the absence of added nitrogen and phosphorus (Figure 22) little change in the saturate profile from the control profile (Figure 21) was noted in the oil recovered from the Peabody Creek and Pt. Partridge incubations. Changes were clearly apparent in the saturate fraction of the oil recovered from the E. Fidalgo incubation.

The results of this latter study show that changes take place in the aromatic fraction, but not the saturate fraction, in the absence of added nitrogen and phosphorus as a result of microbial activity in the Peabody Creek and Pt. Partridge water samples. In contrast, the microbes present in the E. Fidalgo water column samples brought about greater changes in the saturate than in the aromatic fraction of Prudhoe Bay oil without the addition of nitrogen and phosphorus. A comparison of the capillary G.C. data in Figures 19, 20, 21 and 22 indicates that the degradation of the biodegradable components of the aromatic fraction is less dependent on nitrogen and phosphorus supplementation than is the degradation of the saturate fraction of Prudhoe Bay oil.

Role of Fungi and Yeasts

Data on the number of colony forming units, their genera, and oil-degrading capability as measured by changes in the G.C. profile of the n-alkane and isoprenoid profile of recovered oil after 30 days incubation at 20°C are presented in Tables 24, 25, 26, 27 and 28 for sites studied in northern Puget Sound and in Tables 29 and 30 for the Pt. Angeles area.

The data presented in Table 24 were obtained by "in situ" plating of water column material and those in Table 25 by plating the water column and beach samples in the laboratory. Since water plated "in situ" yielded fungi (from samples taken at Samish Island and Pt. Partridge) whereas the laboratory platings did not, all subsequent water samples were plated "in situ". Because of logistic problems a similar comparison was not carried out using beach or sub-tidal samples. Therefore these samples were always plated in the laboratory. The data also show that more fungal colony forming units were isolated from E. Fidalgo samples and that isolates showing maximum oil-degrading activity were obtained from these samples.

A comparison of the growth (numbers and types) of fungal colony forming units on a basal marine agar containing streptomycin (100 μg/ml) and penicillin G (30 units/ml), and Sabouraud's agar (plus marine salts) was carried out on these samples. The types of fungal colony forming units growing on both media were similar; however the basal marine agar supported the growth of more bacteria than did Sabouraud's. Therefore Sabouraud's agar was used for all attempts at the isolation of fungal colony forming units. In order to minimize the growth of bacteria on subsequent platings, antibiotics were included in the saline Sabouraud's agar (Appendix).

TABLE 24. OIL BIODEGRADATIVE ACTIVITY[a] (20°C) OF FUNGI ISOLATED[b] BY PLATING "IN SITU" WATER COLUMN SAMPLES - NORTHERN PUGET SOUND (AUGUST 20-21, 1978).

Site	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
		N	S	P	I
Samish Island	16	Cladosporium(9)[d] Drechslera(1) Trysanophora(1) Unidentified(4)	Unidentified(1)		
E. Fidalgo	10	Cladosporium(4) Unidentified(5)			Aspergillus(1)
Pt. Partridge	3	Cladosporium(1) Unidentified(2)			

[a] based on n-alkane profile of recovered oil

[b] colony forming units growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

TABLE 25. OIL BIODEGRADATIVE ACTIVITY[a] (20°C) OF FUNGI ISOLATED[b] FROM LABORATORY PLATING OF WATER AND BEACH MATERIAL-NORTHERN PUGET SOUND (AUGUST 20-21, 1978).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Samish Island	water	0[d]				
	beach	14	Aureobasidium(1)[e] Cladosporium(1) Penicillium(1) Unidentified(10)	Unidentified(1)		
E. Fidalgo	water	7	Cladosporium(1) Trichoderma(1) Unidentified(4)			Penicillium(1)
	beach	19	Gliocladium(1) Penicillium(1) Stachybotrys(1) Trichoderma(1) Verticillium(1) Unidentified(5)	Penicillium(1)	Penicillium(1) Unidentified(1)	Penicillium(6)
Pt. Partridge	water	0[d]				
	beach	0[d]				

[a] based on n-alkane profile of recovered oil

[b] colony forming units growing on Sabouraud's agar

[c] see Figure 3

[d] no colony forming units isolated under experimental conditions used

[e] Genus (number of isolates tested)

TABLE 26. OIL BIODEGRADATIVE ACTIVITY[a] (8°C) OF FUNGI ISOLATED[b] BY ENRICHMENT PROCEDURE FROM WATER AND BEACH MATERIAL-NORTHERN PUGET SOUND (AUGUST 20-21, 1978).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Samish Island	water	6	Cladosporium(1)[d] Unidentified(5)			
	beach	5	Unidentified(2)		Penicillium(2)	
E. Fidalgo	water	2	Cladosporium(1) Unidentified(1)			
	beach	4	Unidentified(2)			Paecilomyces(1) Penicillium(1)
Pt. Partridge	water	0[e]				
	beach	7	Acremonium(1) Cladosporium(1) Unidentified(3)			Beauveria(1) Penicillium(1)

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[a] based on n-alkane profile of recovered oil

[b] colony forming units growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

[e] no colony forming units isolated under experimental conditions used

TABLE 27. OIL BIODEGRADATIVE ACTIVITY[a] (20°C) OF FUNGI ISOLATED[b] FROM WATER AND BEACH MATERIAL-NORTHERN PUGET SOUND (NOVEMBER 21-22, 1978).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Samish Island	water	4	Unidentified(3)[d]			Penicillium(1)
	beach	1	Unidentified(1)			
E. Fidalgo	water	3	Unidentified(2)		Unidentified(1)	
	beach	12	Unidentified(6)	Unidentified(1)	Unidentified(1)	Penicillium(4)
Pt. Partridge	water	1	Penicillium(1)			
	beach	1	Unidentified(1)			

---

[a] based on n-alkane profile of recovered oil

[b] colony forming units (water samples plated "in situ"; beach samples plated in laboratory) growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

TABLE 28. OIL BIODEGRADATIVE ACTIVITY[a] (8°C) OF FUNGI ISOLATED[b] BY ENRICHMENT PROCEDURE FROM WATER AND BEACH MATERIAL-NORTHERN PUGET SOUND (NOVEMBER 21-22, 1978).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Samish Island	water	1	Unidentified(1)[d]			
	beach	1	Unidentified(1)			
E. Fidalgo	water	2	Verticillium(1)			Penicillium(1)
	beach	4	Fusarium(1)		Unidentified(1)	Unidentified(1)
Pt. Partridge	water	0[e]				
	beach	1	Unidentified(1)			

[a] based on n-alkane profile of recovered oil

[b] colony forming units growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

[e] no colony forming units isolated under experimental conditions used

TABLE 29. OIL BIODEGRADATIVE ACTIVITY[a] (20°C) OF FUNGI ISOLATED[b] FROM WATER AND BEACH MATERIAL - PT. ANGELES AREA (OCTOBER 1-3, 1978).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Freshwater Bay	water	3	Geotrichum(1)[d] Unidentified(2)			
	beach	4	Geotrichum(1) Trichoderma(1) Unidentified(1)	Unidentified(1)		
	sub-tidal sediment	8	Unidentified(5)	Aspergillus(1) Penicillium(1)	Penicillium(1)	
Pt. Angeles-Ediz Hook (Pilot Station)	water	0[e]				
	beach	11	Acremonium(1) Cladosporium(1) Fusarium(1) Geotrichum(1) Penicillium(2) Vlocladium(1) Unidentified(1)	Unidentified(1)		
	sub-tidal		Acremonium(1) Unidentified(1)			Penicillium(1)
Pt. Angeles-Southside (marina)	water	3	Verticillium(1)			Penicillium(1) Unidentified(1)

TABLE 29. continued....

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Pt. Angeles-Southside (Peabody Creek)	water	12	Cladosporium(2) Fusarium(1) Geotrichum(1) Unidentified(4)		Unidentified(1)	Penicillium(3)
	beach	8	Unidentified(8)			
	sub-tidal	8	Trichoderma(2)	Gliomastix(1) Penicillium(1)	Penicillium(1)	Aspergillus(1) Penicillium(2)
Pt. Angeles-Southside (Red Lion Inn)	water	4	Unidentified(4)			
Green Point (west of cavern)	water	2	Aureobasidium(1) Unidentified(1)			
	beach	15	Drechslera(1) Unidentified(9)	Penicillium(1)		Penicillium(2) Verticillium(1) Unidentified(1)
Green Point (west of crevice)	water	2	Unidentified(2)			
Dungeness Spit (2nd navigation marker)	water	2	Unidentified(2)			
	beach	10	Cladosporium(1) Penicillium(1) Unidentified(6)			Penicillium(1) Unidentified(1)

[a] based on n-alkane profile of recovered oil

[b] colony forming units (water samples plated "in situ"; beach samples in laboratory) growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

[e] no colony forming units isolated under experimental conditions used.

TABLE 30. OIL BIODEGRADATIVE ACTIVITY[a] (20°C) OF FUNGI ISOLATED[b] FROM WATER AND BEACH SAMPLES - PT. ANGELES AREA (JANUARY 14-16, 1979).

Site	Sample	Total Isolates	Status of G.C. Profile of Recovered Oil[c]			
			N	S	P	I
Freshwater Bay	water	3	Unidentified(3)[d]			
	beach	1	Unidentified(1)			
Pt. Angeles Southside (Peabody Creek)	water	10	Penicillium(1) Unidentified(9)			
	beach	6	Unidentified(5)			Unidentified(1)
Dungeness Spit (2nd navigation marker)	water	0[e]				
	beach	2				Penicillium(2)

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[a] based on n-alkane profile of recovered oil

[b] colony forming units (water samples plated "in situ"; beach samples in laboratory) growing on Sabouraud's agar

[c] see Figure 3

[d] Genus (number of isolates tested)

[e] no colony forming units isolated under experimental conditions used

The water and beach samples which were plated directly onto Sabouraud's agar were also subjected to our enrichment procedure. Data on the fungal colony forming units isolated by this procedure are presented in Table 26. This technique resulted in the recovery of fungal colony forming units from all beach samples from Samish Island and E. Fidalgo water column samples, but not from the Pt. Partridge water. All enrichments, even though the pH was in the range of 4 to 5, contained readily detectable levels of bacterial contaminants which complicated the purification of the fungal colony forming units present.

None of the 93 fungi isolated from the August, 1979 samples taken in northern Puget Sound were able to use the isoprenoids present in Prudhoe Bay oil. Twelve fungi, *Aspergillus* (1), *Beauveria* (1), *Paecilomyces* (1) and *Penicillium* (9) were able to completely utilize the n-alkanes; 4 fungi, 3 *Penicillium* and 1 unidentified species, were able to partially modify the n-alkanes; 1 *Penicillium* and 2 unidentified fungi were able to selectively utilize the n-alkanes present in Prudhoe Bay oil under the experimental conditions used.

The fungal colony forming units isolated from the November, 1978 samples obtained from northern Puget Sound and their oil-degrading capability are presented in Tables 27 and 28. The results are similar to those obtained in the August samples. Thirty-one fungal colony forming units were isolated by the plating and enrichment techniques (Tables 27 and 28). Seven of these isolates degraded all the n-alkanes, 3 partially removed them and 1 showed a selective utilization pattern. As noted for the August samples, members of the Penicillium genus predominate among those fungi capable of utilizing the n-alkanes present in Prudhoe Bay oil.

Data on the isolation of fungal colony forming units from the Pt. Angeles area are presented in Table 29 and Table 30. The results from the October sampling (Table 29) include data on isolations from water column, beach and sub-tidal sediment samples. Ninety-five isolates were obtained, 15 of which utilized all the n-alkanes, 3 partially removed them and 7 showed a selective utilization profile. As previously observed, isolates from the genus Penicillium predominate among those fungi capable of utilizing the n-alkanes present in Prudhoe Bay oil.

The addition of antibiotics (streptomycin 100 μg/ml and penicillin G (60,000 units/L) to enrichment cultures set up from Pt. Angeles samples taken in January, 1979 was not effective in controlling the growth of bacteria in enrichments. As the types, numbers and oil-degrading activities obtained from these enrichments were similar to the data already presented, they are not included in this report.

A total of 237 fungi were isolated by direct plating and enrichment techniques from water, beach and sub-tidal sediments of northern Puget Sound and the Pt. Angeles area of the Strait of Juan de Fuca. The majority of the isolates (75.5%) were not able to utilize the n-alkanes present in Prudhoe Bay oil for growth. Approximately one-quarter of them were able to bring about changes in the G.C. profile of the saturate fraction of this oil: 4.6% brought about selective changes; 4.2% partially degraded the n-alkanes present; 15.6% completely utilized the n-alkanes. None however were able to utilize the isoprenoids present in Prudhoe Bay oil.

The data previously reported on oil degradation were obtained by incubation at 20°C for 30 days. A similar series of studies was carried out with incubation at 8°C for 60 days. The majority of the 237 isolates (83%) did not utilize the n-alkanes in Prudhoe Bay oil for growth at this temperature. Of the 17% which did modify the n-alkane profile only 3.4% were able to completely utilize these components, 7.2% partially removed them and 6.4% selectively utilized them. Only one isolate was able to bring about greater changes in the n-alkane components at 8°C than at 20°C. The ability of these fungi to grow on Prudhoe Bay oil as indicated by changes in the n-alkane and isoprenoid profiles, is much less at 8°C (in spite of a longer incubation time), than at 20°C. The incidence of n-alkane-utilizing fungi isolated (with one exception - the August "in situ" water platings from northern Puget Sound) is in the range of 17±5% for all sites studied.

Yeasts were always present in samples from all sites, particularly in those platings of enrichment cultures. Seventy-four yeasts were screened for their ability to grow on the n-alkanes present in Prudhoe Bay oil and only 3 completely utilized the n-alkanes. These were tentatively identified (via the Analytab Products-API$_{20}$ Clinical Yeast System for in Vitro Diagnostic Use; and by morphology studies) as being strains of *Candida tropicalis*, *Candida marina* and *Leucosporidium antarctica*. None of the other yeasts were capable of bringing about any changes in the n-alkane profile of Prudhoe Bay oil. As the basal medium used for screening of oil-degrading capability does not contain vitamins, 54 of the yeast isolates, including the 3 degraders, were grown on a medium containing Bacto Yeast Nitrogen Base with Prudhoe Bay oil as the sole carbon source. The G.C. profile results were identical to those obtained using the basal agar, thus the presence of vitamins does not improve the oil-degrading capability of these marine yeast isolates.

The results presented in Table 31 indicate that the high aeration and cultural conditions obtained by liquid shake culture do not enhance oil degradation by the fungi and yeasts tested.

Microbial Numbers and Bacterial Taxonomy

The numbers of bacterial and yeast-fungal colony forming units found in water and beach samples from northern Puget Sound sites obtained in August 1978 are presented in Table 32. There was a slight increase in the number of bacterial colony forming units obtained in water samples that had been transported to the laboratory before plating compared with the numbers obtained for "in situ" plating. Such differences were not noted with data obtained for the number of yeast-fungal colony forming units. In order to minimize changes in the number of colony forming units all water samples

TABLE 31. EFFECT OF CULTURE TECHNIQUE ON THE UTILIZATION OF THE N-ALKANES IN PRUDHOE BAY OIL.

Isolate	Status of G.C. Profile of Recovered Oil[a]	
	Static Culture[b]	Shake Culture[c]
Aspergillus sp.	I	I
Aspergillus sp.	I	S
Beauveria sp.	I	I
Gliomastix sp.	S	S
Paecilomyces sp.	I	P
Penicillium sp.	P	P
Penicillium sp.	I	P
Penicillium sp.	P	S
Penicillium sp.	I	S
Penicillium sp.	S	S
Verticillium sp.	I	P
Candida marina	S	S
Candida tropicalis	I	S
Leucosporidium antarctica	I	P
Rhodotorula sp.	N	N

[a] see Figure 3

[b] slant culture technique

[c] rotary shaker @ 240 RPM for 30 days at 20°C

TABLE 32. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER AND BEACH SAMPLES - NORTHERN PUGET SOUND SITES (AUGUST 20-21, 1978).

Site	Sample	Bacteria[a]		Yeast and Fungi[a]			
				"in situ"		in laboratory	
		"in situ"	in laboratory	Sabouraud's medium	Basal medium	Sabouraud's medium	Basal medium
Samish Island	water	$1.77(\pm0.19)\times10^{4}$	$5.7(\pm0.6)\times10^{4}$	$6(\pm2)\times10^{1}$	$4(\pm2)\times10^{1}$	$4(\pm5)\times10^{0}$	$5(\pm5)\times10^{1}$
	beach	—[b]	$6.3(\pm1.1)\times10^{6}$	—	—	$2.8(\pm0.5)\times10^{3}$	$2.0(\pm0.3)\times10^{3}$
E. Fidalgo	water	$7.1(\pm1.2)\times10^{4}$	$1.61(\pm0.25)\times10^{5}$	$1(\pm1)\times10^{1}$	$2(\pm2)\times10^{1}$	$3(\pm1)\times10^{1}$	$6(\pm3)\times10^{1}$
	beach	—	$8.9(\pm1.2)\times10^{5}$	—	—	2.5×10^{3}	$1.9(\pm0.5)\times10^{3}$
Pt. Partridge	water	$1.28(\pm0.08)\times10^{4}$	$1.05(\pm0.11)\times10^{5}$	$4(\pm5)\times10^{0}$	$2(\pm3)\times10^{1}$	$2(\pm4)\times10^{0}$	$2(\pm4)\times10^{0}$
	beach	—	$4.8(\pm0.6)\times10^{4}$	—	—	$< 10^{1}$	$< 10^{1}$

[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach material from 5 replicate plates

[b] not plated under these conditions

were plated "in situ". No significant differences were noted in the yeast-fungal counts obtained on Sabouraud's agar (salinity adjusted) and those obtained on the basal marine agar. However fewer bacterial colonies appeared on Sabouraud's medium, therefore it was routinely used for the enumeration of yeast-fungal colony forming units. Low levels of the antibiotics streptomycin and penicillin (see Appendix for composition) to minimize bacterial growth were also incorporated into Sabouraud's agar.

The data on colony forming units for the November, 1978 and April, 1979 samplings of the northern Puget Sound sites are presented in Tables 33 and 34 respectively. The numbers of bacteria and yeast-fungal colony forming units were similar for the three sampling times investigated. However, the numbers of bacteria found were 2 to 3 orders of magnitude greater than those obtained for yeast-fungi.

The generic composition of the bacterial populations present in E. Fidalgo and Pt. Partridge water and beach samples obtained in April, 1979 before and after enrichment with Prudhoe Bay oil and added nitrogen and phosphorus, are presented in Table 35. The water column samples have a more diverse flora than do the beach samples before enrichment. Members of the Flavobacterium and Pseudomonas genera predominate in enriched, oil-degrading populations.

Table 36 presents the generic composition of enriched, oil-degrading populations obtained from cobble stones from E. Fidalgo and Pt. Partridge. Comparing these results with data obtained from water and beach samples from the respective sites, the composition of the cobble populations varies considerably although members of the Flavobacterium and Pseudomonas genera predominate in 3 of the 6 populations studied.

The numbers of bacterial and yeast-fungal colony forming units found in water, beach and sub-tidal samples from the Pt. Angeles area are presented in Table 37. The numbers of bacterial colony forming units are 2 to 3 orders of magnitude greater than those obtained for yeast-fungal colony forming units. Very little difference occurs between numbers of bacterial or yeast-fungal colony forming units from pristine areas (e.g. as in the Dungeness Spit area) and counts obtained from Pt. Angeles harbor sites (e.g. Peabody Creek). The generic compositions (Table 38) of the bacterial populations found in the sub-tidal sediments show a relatively high percentage of colonies which did not grow on transfers.

The numbers of bacterial and yeast-fungal colony forming units found in the January 1979 samples from Freshwater Bay, Pt. Angeles - Peabody Creek and Dungeness Spit #2 are presented in Table 39. The counts are similar to those obtained from the October samples.

Data on the number of colony forming units in water, beach and subtidal sediment samples (June, 1979) taken from Pt. Angeles harbor (Ediz Hook-pilot station and Peabody Creek) and Dungeness Spit are presented in

TABLE 33. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER AND BEACH SAMPLES - NORTHERN PUGET SOUND SITES (NOVEMBER 21-22, 1978).

Site	Sample	Bacteria[a]		Yeast and Fungi[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Samish Island	water	$3.28(\pm0.30)x10^{3}$	$1.36(\pm0.24)x10^{4}$	$8(\pm8)x10^{0}$	—
	beach	—[c]	$1.79(\pm0.45)x10^{5}$	—	$2.1(\pm4.6)x10^{1}$
E. Fidalgo	water	$1.39(\pm0.09)x10^{4}$	$3.82(\pm0.30)x10^{4}$	$2(\pm2)x10^{1}$	—
	beach	—	$2.90(\pm0.31)x10^{5}$	—	$5.8(\pm2.5)x10^{2}$
Pt. Partridge	water	$2.17(\pm0.35)x10^{3}$	$2.22(\pm0.26)x10^{3}$	$2(\pm5)x10^{0}$	—
	beach	—	$5.3(\pm0.6)x10^{4}$	—	$4.1(\pm5.6)x10^{1}$

[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach material from 5 replicate plates

[b] Sabouraud's medium

[c] not plated under these conditions

TABLE 34. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER AND BEACH SAMPLES - NORTHERN PUGET SOUND SITES (APRIL 2-3, 1979).

Site	Sample	Bacteria[a]		Yeast and Fungi[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Samish Island	water	$3.3(\pm0.7)\times10^{3}$	—[c]	$3(\pm2)\times10^{1}$	—
	beach	—	$5.0(\pm0.2)\times10^{4}$	—	$7(\pm11)\times10^{1}$
E. Fidalgo	water	$1.8(\pm0.6)\times10^{4}$	$4.8(\pm1.0)\times10^{4}$	$2(\pm1.3)\times10^{1}$	—
	beach	—	$5.3(\pm0.6)\times10^{5}$	—	$1(\pm0.2)\times10^{3}$
Pt. Partridge	water	$2.2(\pm0.6)\times10^{3}$	$3.6(\pm0.6)\times10^{5}$	$>10^{1}$	—
	beach	—	$2.2(\pm0.5)\times10^{4}$	—	$>10^{1}$

[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach material from 5 replicate plates

[b] Sabouraud's medium

[c] not plated under these conditions

TABLE 35. GENERIC COMPOSITION OF BACTERIAL POPULATIONS IN E. FIDALGO AND PT. PARTRIDGE BEFORE AND AFTER ENRICHMENT IN THE PRESENCE OF ADDED NITROGEN AND PHOSPHORUS (8°C) (APRIL 2-3, 1979).

Genus	% Generic Composition							
	E. Fidalgo				Pt. Partridge			
	Water		Beach		Water		Beach	
	Before[a]	After[b]	Before	After	Before	After	Before	After
Acinetobacter sp.	-[c]	-	-	5.0	-	-	-	
Aeromonas sp.	-	-	-	0.2	2.9	-	-	6.0
Alcaligenes sp.	21.4	13.4	31.4	2.2	20.9	0.2	23.4	15.2
Coryneforms	25.8	-	-	3.0	13.4	-	-	12.0
Cytophaga sp.	-	-	0.8	-	3.0	-	3.7	-
Flavobacterium sp.	28.1	54.2	50.2	72.5	17.9	0.4	61.7	65.0
Pseudomonas sp.	3.4	8.1	8.0	2.3	4.5	98.6	0.9	1.8
Vibrio sp.	-	-	-	-	14.9	-	-	-
Unidentified[d]	14.6	24.5	9.2	14.9	16.4	0.6	10.3	-
Non-Transferable[e] Colonies	6.7	-	0.4	-	6.0	0.2	-	-

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[a] before enrichment (i.e. original samples)

[b] after enrichment with nitrogen, phosphorus and Prudhoe Bay oil present

[c] no colonies present on dilution plate used to assess population composition

[d] not classifiable on the basis of the characteristics used

[e] colonies which did not grow on transfer

TABLE 36. GENERIC COMPOSITION OF BACTERIAL POPULATIONS IN WATER, BEACH AND COBBLE SAMPLES FROM E. FIDALGO AND PT. PARTRIDGE (APRIL 2-3, 1979) AFTER ENRICHMENT WITH PRUDHOE BAY AND ADDED NITROGEN AND PHOSPHORUS.

Genus	% Generic Composition									
	E. Fidalgo					Pt. Partridge				
	Water	Wet[a] Cobble	Damp[b] Cobble	Dry[c] Cobble	Beach	Water	Wet Cobble	Damp Cobble	Dry Cobble	Beach
Acinetobacter sp.	-[d]	-	-	63.0	5.0	-	-	-	0.4	-
Aeromonas sp.	-	2.8	-	-	0.2	-	3.2	-	-	6.0
Alcaligenes sp.	13.4	25.8	-	22.6	2.2	0.2	24.2	54.9	13.2	15.2
Bacillus sp.	-	0.9	-	-	-	-	-	-	-	-
Coryneforms	-	5.6	1.8	3.8	3.0	-	3.2	0.5	29.1	12.0
Cytophaga sp.	-	1.8	-	0.3	-	-	1.3	17.5	-	-
Flavobacterium sp.	54.2	19.5	73.0	2.6	72.5	0.4	0.9	19.5	0.6	65.0
Pseudomonas sp.	8.1	13.6	9.9	7.3	2.3	98.6	66.9	7.7	46.5	1.8
Vibrio sp.	-	-	-	0.3	-	-	0.3	-	-	-
Unidentified[e]	24.5	19.2	14.5	0.3	14.9	0.6	-	-	10.4	-
Non-Transferable[f] Colonies	-	10.8	0.9	-	-	0.2	-	-	-	-

[a] closest to tide
[b] mid-way between tide and high tide line
[c] dry; just below high tide line
[d] no colonies present on dilution plate used to assess population composition
[e] not classifiable on the basis of the characteristics used
[f] colonies which did not grow on transfer

TABLE 37. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER, BEACH AND SUB-TIDAL SAMPLES - PT. ANGELES AREA (OCTOBER 1-3, 1978).

Site	Sample	Bacteria[a]		Fungi and Yeasts[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Fresh water Bay	water	$5.5(\pm2.9)x10^{4}$	$4.5(\pm0.9)x10^{4}$	$1.1(\pm0.4)x10^{2}$	$7(\pm3)x10^{1}$
	beach	____[c]	$4.3(\pm0.9)x10^{5}$	____	$4(\pm2)x10^{2}$
	sediment[d]	____	$1.0(\pm0.2)x10^{6}$	____	$4(\pm3)x10^{2}$
Pt. Angeles-Ediz Hook (Pilot Station)	water	$8.1(\pm1.1)x10^{3}$	$3.9(\pm0.6)x10^{4}$	$3(\pm1)x10^{1}$	$6(\pm5)x10^{0}$
	beach	____	$4.7(\pm1.1)x10^{6}$	____	$1.2(\pm0.4)x10^{3}$
	sediment	____	$7.1(\pm2.3)x10^{5}$	____	$3(\pm1)x10^{2}$
Pt. Angeles-Southside (Marina)	water	$4.0(\pm1.2)x10^{4}$	____	$2(\pm1)x10^{1}$	____
Pt. Angeles-Southside (Peabody Creek)	water	$4.6(\pm0.7)x10^{4}$	$3.83(\pm0.13)x10^{4}$	$6(\pm2)x10^{1}$	$3(\pm2)x10^{1}$
	beach	____	$1.22(\pm0.28)x10^{6}$	____	$5(\pm2)x10^{2}$
	sediment	____	$5.9(\pm1.3)x10^{5}$	____	$1.5(\pm0.3)x10^{3}$
Pt. Angeles-Southside (Red Lion Inn)	water	$2.08(\pm0.13)x10^{4}$	____	$2(\pm1)x10^{1}$	____

Site	Sample	Bacteria[a]		Fungi and Yeasts[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Green Point (west by cavern)	water	1.38(±0.11)x10^{4}	4.5(±0.8)x10^{4}	6(±3)x10^{1}	1(±1)x10^{1}
	beach	___	1.54(±0.41)x10^{7}	___	7.6(±0.6)x10^{3}
Green Point (west of crevice)	water	3.1(±1.4)x10^{4}	___	7(±1)x10^{1}	___
Dungeness (by 2nd navigation marker)	water	7.8(±1.6)x10^{3}	2.9(±0.7)x10^{4}	3(±1)x10^{1}	5(±3)x10^{1}
	beach	___	2.34(±0.84)x10^{6}	___	1.4(±0.2)x10^{3}

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[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach or sub-tidal sediment material from 5 replicate plates

[b] Sabouraud's medium

[c] not plated under these conditions

[d] sub-tidal sediment

TABLE 38. GENERIC COMPOSITION OF BACTERIAL POPULATIONS IN SUB-TIDAL SEDIMENTS FROM THE PT. ANGELES AREA (OCTOBER 1-3, 1978).

	% Generic Composition		
		Pt. Angeles	
Genus	Freshwater Bay	Ediz Hook (Pilot Station)	Peabody Creek
Acinetobacter sp.	2.0	12.0	7.4
Aeromonas sp.	5.9	-[a]	-
Alcaligenes sp.	3.1	21.8	1.7
Bacillus sp.	0.6	0.4	5.7
Coryneforms	0.3	5.6	2.3
Cytophaga sp.	0.3	-	0.6
Flavobacterium sp.	9.0	16.7	1.7
Pseudomonas sp.	9.8	30.8	17.6
Vibrio sp.	2.0	-	3.4
Unidentified[b]	30.2	0.4	2.8
Non-Transferable[c] Colonies	37.2	12.4	56.8

[a] no colonies present on dilution plate used to assess population composition

[b] not classifiable on the basis of the characteristics used

[c] colonies which did not grow on transfer

TABLE 39. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER AND BEACH SAMPLES - PT. ANGELES AREA (JANUARY 14-16, 1979).

Site	Sample	Bacteria[a]		Fungi and Yeasts[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Freshwater Bay	water	$2.3(\pm0.16)\times10^4$	$1.12(\pm0.10)\times10^5$	$6(\pm1.3)\times10^0$	___
	beach	___[c]	$4.6(\pm0.6)\times10^5$	___	$2.1(\pm4.7)\times10^1$
Pt. Angeles-Southside (Peabody Creek)	water	$1.55(\pm0.17)\times10^5$	$3.19(\pm0.31)\times10^5$	$2.6(\pm1.1)\times10^1$	___
	beach	___	$3.09(\pm0.26)\times10^5$	___	$4.0(\pm3.2)\times10^2$
Dungeness (by 2nd navigation marker)	water	$1.04(\pm0.14)\times10^5$	$1.72(\pm0.25)\times10^5$	$2(\pm4.5)\times10^0$	___
	beach	___	$6.6(\pm1.3)\times10^4$	___	$1.4(\pm1.4)\times10^2$

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[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach material from 5 replicate plates.

[b] Sabouraud's medium

[c] not plated under these conditions

Table 40. The generic composition of the bacterial populations before and after enrichment from these samples are presented in Table 41 and 42 respectively.

The numbers of colony forming units found in the June 1979 samples are similar to those reported for January, 1979 and October, 1978 samples. Members of the Cytophaga and Flavobacterium genera predominate in unenriched populations (Table 41). The composition of the enriched populations varies markedly although members of the Flavobacterium genus predominate (i.e. greater than 30% of the population) in 6 of the 9 populations studied.

Data in Table 43 show total heterotrophic counts on the basal marine agar used in these studies, a commercial marine agar (Difco 2216) and a commercial medium (Difco TCBS) which is selective for disease-causing vibrios. Similar levels of bacterial colony forming units were obtained on both the basal marine agar and the commercial product (Difco 2216). Very few members of the Vibrio genus (common marine bacteria) were recovered on either of these marine agars. The incidence of vibrios in the samples analyzed is reflected in the numbers of colony forming units growing on TCBS agar. Typical vibrios produce yellow colored colonies when grown on this medium. Such colored colonies formed from 25% to 88% (average 52%) of the colonial counts reported on TCBS agar for water, beach and sediment samples analyzed (Table 43). Thus the actual vibrio count would be approximately one-half of the total number of colonies growing on TCBS agar and could represent up to 5% of the total bacterial colony forming units capable of growth under the conditions used in this study. Transfers of these "presumptive" vibrio isolates readily grew on both marine agars used for determining the number of heterotrophic colony forming units. Therefore the failure to detect these typical marine bacteria in enriched, oil-degrading populations is not due to their inability to grow on basal marine agar but rather to their lack of direct participation in the oil-degrading process.

Oil-Degradation by Duwamish River Samples

The numbers of bacterial and yeast-fungal colony forming units detected in water and inter-tidal sediments from the Duwamish River are presented in Table 44. The numbers of bacteria obtained from water column samples using saline basal marine agar and non-saline plate count agar are similar and are of the same order of magnitude as reported for water column samples in northern Puget Sound and the Pt. Angeles area. The number of yeast-fungal colony forming units in water column samples are also similar to those reported for northern Washington waters. The numbers of microorganisms found in the Duwamish inter-tidal sediments are similar to those found in such materials from the other sites investigated in this study.

The oil-degrading capability of these microbial populations with and without nitrogen and phosphorus supplementation, and data on the chemical analyses of samples are presented in Table 45. Very high levels of

TABLE 40. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER, BEACH AND SUB-TIDAL SAMPLES - PT. ANGELES AREA (JUNE 18-20, 1979).

Site	Sample	Bacteria[a]		Fungi and Yeasts[a,b]	
		"in situ"	in laboratory	"in situ"	in laboratory
Pt. Angeles- Ediz Hook (Pilot Station)	water	$2.88(\pm0.26)x10^{4}$	$5.0(\pm0.74)x10^{4}$	$2(\pm4.5)x10^{0}$	___
	beach	___[c]	$2.3(\pm0.45)x10^{5}$	___	$2.7(\pm1.1)x10^{4}$
	sediment[d]	___	$3.27(\pm0.19)x10^{5}$	___	$3.0(\pm3.1)x10^{2}$
Pt. Angeles- Southside (Peabody Creek)	water	$8.1(\pm1.1)x10^{4}$	$1.52(\pm0.24)x10^{5}$	$3(\pm1.8)x10^{1}$	___
	beach	___	$3.93(\pm0.39)x10^{7}$	___	$4.6(\pm1.3)x10^{3}$
	sediment	___	$7.5(\pm1.7)x10^{5}$	___	$1.7(\pm0.2)x10^{3}$
Dungeness (by 2nd navigation marker)	water	$5.0(\pm1.2)x10^{4}$	$3.4(\pm0.5)x10^{4}$	$> 10^{1}$	___
	beach	___	$2.2(\pm0.6)x10^{4}$	___	$> 10^{1}$
	sediment	___	$4.2(\pm1.0)x10^{5}$	___	$> 10^{1}$

[a] colony forming units (± 1 standard deviation) per mL of water or g (dry weight) of beach or sub-tidal sediment material from 5 replicate plates.

[b] Sabouraud's medium

[c] not plated under these conditions

[d] sub-tidal sediment

TABLE 41. GENERIC COMPOSITION OF BACTERIAL POPULATIONS IN WATER, BEACH AND SUB-TIDAL SAMPLES FROM THE PT. ANGELES AREA (JUNE 18-20, 1979) BEFORE ENRICHMENT AT 8°C WITH PRUDHOE BAY OIL AND NITROGEN AND PHOSPHORUS.

Genus	% Generic Composition								
	Pt. Angeles Area								
	Ediz Hook (Pilot Station)			Peabody Creek			Dungeness Spit #2		
	Water	Beach	Sediment (sub-tidal)	Water	Beach	Sediment (sub-tidal)	Water	Beach	Sediment (sub-tidal)
Acinetobacter sp.	4.0	21.8	0.9	-[a]	22.2	-	-	-	1.3
Aeromonas sp.	0.5	-	0.3	-	0.6	6.9	-	-	11.5
Alcaligenes sp.	1.5	2.7	70.5	4.8	1.4	47.4	-	-	-
Bacillus sp.	-	-	3.0	-	-	1.7	-	-	-
Coryneforms	-	-	3.1	-	-	-	-	-	-
Cytophaga sp.	5.0	38.2	11.4	46.8	17.4	16.6	61.8	41.4	8.9
Flavobacterium sp.	72.2	19.1	3.3	40.7	14.4	10.3	31.8	51.9	70.7
Pseudomonas sp.	-	4.6	-	-	-	1.7	-	-	-
Unidentified[b]	1.0	13.6	0.4	7.7	0.7	9.7	6.5	6.7	5.7
Non-Transferable Colonies[c]	15.9	-	7.1	-	43.3	5.7	-	-	1.9

[a] no colonies present on dilution plate used to assess population composition

[b] not classifiable on the basis of the characteristics used

[c] colonies which did not grow on transfer

TABLE 42. GENERIC COMPOSITION OF BACTERIAL POPULATIONS IN WATER, BEACH AND SUB-TIDAL SAMPLES FROM THE PT. ANGELES AREA (JUNE 18-20, 1979) AFTER ENRICHMENT AT 8°C WITH PRUDHOE BAY OIL AND NITROGEN AND PHOSPHORUS.

Genus	% Generic Composition								
	Pt. Angeles Area								
	Ediz Hook (Pilot Station)			Peabody Creek			Dungeness Spit #2		
	Water	Beach	Sediment (sub-tidal)	Water	Beach	Sediment (sub-tidal)	Water	Beach	Sediment (sub-tidal)
Aeromonas sp.	-[a]	-	-	-	-	-	-	27.5	6.6
Alcaligenes sp.	61.0	12.7	-	45.6	-	7.1	21.1	-	23.1
Coryneforms	-	3.5	7.5	0.8	-	5.3	-	-	1.7
Cytophagas sp.	0.9	0.1	19.1	4.7	-	18.6	8.1	11.1	-
Flavobacterium sp.	37.3	83.1	61.0	10.2	-	36.3	23.2	61.4	32.2
Pseudomonas sp.	-	-	-	18.9	-	-	6.0	-	26.5
Unidentified[b]	0.8	0.4	10.2	-	-	1.8	-	-	9.9
Non-Transferable[c] Colonies[c]	-	0.3	2.3	19.7	-	31.0	41.6	-	-

[a] no colonies present on dilution plates used to assess population composition

[b] not classifiable on the basis of the characteristics used

[c] colonies which did not grow on transfer

TABLE 43. TOTAL HETEROTROPHIC COUNT ON BASAL MARINE AGAR, MARINE AGAR (DIFCO-2216) AND TCBS (DIFCO) AGAR IN WATER, BEACH AND SUB-TIDAL SAMPLES FROM PT. ANGELES AREA (JUNE 18-20, 1979).

Site	Sample	Bacterial Colony Forming Units		
		Basal Marine Agar[a]	Marine Agar 2216 (DIFCO)[b]	TCBS Agar[c]
Pt. Angeles- (Ediz Hook, Pilot Station)	water	$7.4(\pm 1.2)x10^{4}$	$5.0(\pm 0.7)x10^{4}$	$2.5(\pm 0.7)x10^{2}$
	beach	$1.5(\pm 0.2)x10^{5}$	$2.3(\pm 0.5)x10^{5}$	$4.0(\pm 0.6)x10^{3}$
	sediment[d]	$1.9(\pm 0.1)x10^{5}$	$3.3(\pm 0.2)x10^{5}$	$7.0(\pm 3.2)x10^{3}$
Pt. Angeles- (Peabody Creek)	water	$8.3(\pm 1.5)x10^{4}$	$1.5(\pm 0.2)x10^{5}$	$1.6(\pm 0.8)x10^{3}$
	beach	$1.3(\pm 0.2)x10^{7}$	$3.9(\pm 0.4)x10^{7}$	$4.7(\pm 1.2)x10^{5}$
	sediment	$5.1(\pm 0.5)x10^{5}$	$7.5(\pm 1.7)x10^{5}$	$6.7(\pm 0.5)x10^{4}$
Dungeness Spit #2	water	$2.9(\pm 0.4)x10^{4}$	$3.4(\pm 0.5)x10^{4}$	$1.8(\pm 0.4)x10^{3}$
	beach	$2.0(\pm 0.5)x10^{4}$	$2.2(\pm 0.6)x10^{4}$	$7.0(\pm 3.8)x10^{2}$
	sediment	$4.3(\pm 0.7)x10^{5}$	$4.2(\pm 1.0)x10^{5}$	$1.4(\pm 2.7)x10^{4}$

[a] see Appendix for composition

[b] Bacto Marine Agar (Difco) for culture and enumeration of heterotrophic marine bacteria

[c] Thiosulfate Citrate Bile Salts Agar (selective medium for isolation of pathogenic vibrios

[d] sub-tidal sediment

TABLE 44. BACTERIAL, YEAST AND FUNGAL COLONY FORMING UNITS - WATER AND SEDIMENT SAMPLES-DUWAMISH RIVER (AUGUST 20-21, 1979).

Site	Sample	Bacteria[a,b]			Yeast[a,b]	Fungi[a,b]
		Marine Agar (Basal)		Plate Count Agar[c]		
		in situ	in laboratory	in laboratory		
Tukwila	water	$7.6(\pm1.7)\times10^{3}$	-[d]	$1.2(\pm0.64)\times10^{4}$	$5(\pm2)\times10^{1}$	$1.5(\pm0.41)\times10^{2}$
	sediment[e]	-	$1.7(\pm0.17)\times10^{6}$	$1.7(\pm0.31)\times10^{6}$	$1.9(\pm0.5)\times10^{3}$	$6.2(\pm0.63)\times10^{3}$
Monroe & 10th Ave.	water	$1.2(\pm0.29)\times10^{4}$	-	$8(\pm1)\times10^{3}$	$2(\pm0.7)\times10^{1}$	$5(\pm3)\times10^{1}$
	sediment	-	$3.0(\pm0.26)\times10^{5}$	$9.8(\pm6.5)\times10^{5}$	$1.8(\pm0.5)\times10^{3}$	$2.0(\pm0.8)\times10^{3}$
Klikitat Ave. (Fisher Mills)	water	$8.3(\pm2.0)\times10^{3}$	-	$6(\pm3)\times10^{3}$	$1(\pm0.5)\times10^{1}$	$3(\pm2)\times10^{1}$
Pacific N.W. Bell Cabel Crossing (Southside)	water	$8.8(\pm1.6)\times10^{3}$	-	$3(\pm0.6)\times10^{3}$	$0.4(\pm0.6)\times10^{0}$	$2(\pm1)\times10^{1}$

---

[a] colony forming units (± 1 standard deviation) per mL of liquid or per g (wet weight) of sediment

[b] water samples plated "in situ" and sediments plated in the laboratory

[c] standard plate count agar (i.e. non-saline)

[d] not plated under these conditions

[e] inter-tidal sediment

TABLE 45. G.C. PROFILE OF PENTANE EXTRACT OF RECOVERED PRUDHOE BAY OIL AFTER 28 DAYS INCUBATION AT 8°C WITH DUWAMISH RIVER WATER OR SEDIMENT SAMPLES WITH AND WITHOUT NITROGEN AND PHOSPHORUS SUPPLEMENTATION AND THE CHEMICAL ANALYSIS OF SAMPLES - (AUGUST 20-21, 1979).

Site	Sample	G.C. Profile		Nitrogen (µg)[a]		Total orthophosphate (µg)[a]	Total Organic Carbon (mg/L)	Greases and oils (µg)[a]	Salinity (o/oo)
		+N,P	-N,P	NO_3^-	NH_4^+				
Tukwila	water	I[b]	N	380	150	38	8	200	ND[c]
	sediment[d]	C	S	3.9	13	77	-[e]	0.58	-
119 St. & Duwamish Rv.	water	I	S	480	>1000	440	10	310	ND
	sediment	C	P	5.0	16	280	-	1.2	-
Monroe & 10th Ave.	water	C	S	460	670	220	9	300	12.0
	sediment	C	N	3.3	6	120	-	0.2	-
S.W. Dakota & Duwamish Rv.	water	I	N	380	570	150	8	190	17.8
	sediment	I	P	4.0	8.0	39	-	0.51	-
Klikitat Ave. (Fisher Mills)	water	C	N	330	550	170	-	310	18.8
Pacific N.W. Bell Cable-Crossing	water	I	N	280	200	88	9	290	22.8

[a] µg per L water or mgs per g (wet weight) sediment
[b] see Figure 3
[c] not detectable by analytical methods used
[d] inter-tidal sediment
[e] not analyzed

oil-degrading activity occurred in those samples which received a nitrogen-phosphorus supplement. Without nutrient supplementation 5 of the 10 samples examined were able to bring about readily detectable changes in the n-alkane profile of Prudhoe Bay oil within the 28 day incubation period at 8°C.

SECTION 6

DISCUSSION

Three northern Puget Sound sites were selected to represent the diverse marine environmental conditions found in these waters and were investigated in this study: Samish Island - an area rich in organic matter; E. Fidalgo - a bay area rich in organic matter which is likely to have been contaminated with hydrocarbons (i.e. close proximity to oil refineries); and Pt. Partridge - a pristine area low in organic matter. The sites used in the Pt. Angeles area were selected to provide detailed information on the waters, beaches and sediments within the harbor and were contrasted with more pristine sites found in Freshwater Bay and in the area from Green Point to Dungeness Spit.

The temperature, often a rate-limiting parameter in microbial activities such as oil-degradation (17), of samples taken in this investigation ranged from 4°C to 22°C (Appendix) with beach samples being a few degrees warmer than water column samples. The pH of water column samples ranged from 6.9 to 8.3 (8), while the dissolved oxygen varied from 3.6 to 14.4 ppm and the salinity from 18.2 to 28.3 ppt. All of these physical characteristics are in the range which would support the growth of marine psychrotrophic heterotrophic microbial species.

As long as the physical parameters of an environment are in ranges which support microbial growth, the removal of exogenous carbon sources like oil will be dependent on the nitrogen and phosphorus levels in that environment (4, 5, 29). The phosphorus level, in particular, has been cited (21) as the key nutrient in controlling the rate of oil removal in open oceans whereas other studies (8, 29) indicate that the removal of oil from marine waters is reported to be more sensitive to low levels of nitrogen than phosphorus. The data obtained in this current study confirm the importance of added nutrients (i.e. nitrogen and phosphorus) in accelerating the mineralization of Prudhoe Bay oil by the microbial flora present in these marine environments.

Oil-degrading capability has been reported in terms of a "Degradative Capacity Index". This reflects the microbial flora's ability to remove the n-alkanes and isoprenoids present in the saturate fraction of oils (e.g. Prudhoe Bay). That is, activity is based on the demonstration of changes in the chemical composition of the oil brought about by a biological response (8, 12).

Differences in oil-degrading activity were observed in the psychrotrophic oil-degrading capability of water column and beach samples obtained at each site as well as between samples from different sites (Tables 3 and 4). Higher levels of activity were found in beach material than in the corresponding water column sample and the activity tended to persist on a seasonal basis for a longer period of time in beach samples (Tables 3, 4, 5). The viable counts of beach samples were 1 to 2 and occasionally 3 orders of magnitude greater than those found in the corresponding water column samples (Tables 32, 33, 34, 37, 38, 39, 40). Neither this observation nor the differences noted in generic composition of the component bacterial population (Tables 35, 41, 42) could be correlated with differences in oil-degrading activity. Such oil-degrading activity, as previously reported (8), was more closely associated with proximity to oil refineries (e.g. E. Fidalgo) and to heavy commercial and recreational use (e.g. Pt. Angeles harbor). This latter point is demonstrated (Tables 6, 7) in that samples taken from within the Pt. Angeles harbor area (i.e. Ediz Hook to the site west of Morse Creek) had higher levels of oil-degrading activity than those obtained from the sites east of Morse Creek to Dungeness Spit.

Oil spilled in the marine environment may be incorporated into inter-tidal and sub-tidal sediments. Examination of oil-degrading ability of sections of inter-tidal cores indicates that a marked variation in activity exists between sites (Table 8). For example, oil-degrading activity was found only in the surface centimeter of a core taken at Peabody Creek (Pt. Angeles harbor), whereas activity was uniformly distributed throughout the E. Fidalgo core and was hardly detectable in a core taken on Dungeness Spit. The variation in oil-degrading activity does not correlate with initial viable count of bacteria present in samples nor with the total viable count reached after enrichment with Prudhoe Bay oil and an exogenous source of nitrogen and phosphorus. It is probably related to the types of bacteria present which not only vary from site to site but also from section to section of a sample. The changing incidence of pigmented colony forming units both within and between cores, although not related to oil-degrading capability, illustrates the heterogeneity of the bacterial populations present in these cores.

If oil is spilled in a marine environment the seaweed and cobbles present are likely to be contaminated with oil. Such materials will in all likelihood have an attached (i.e. periphytic) microbial flora (30). The presence of such bacteria with the capability of bringing about chemical changes in Prudhoe Bay oil was demonstrated when enrichments from seaweeds, cobbles and cobble washes in the presence of added nitrogen and phosphorus brought about changes in the composition of Prudhoe Bay oil (Tables 9, 10, 11). The observation that transfers of the initial enrichments were more active than the original enrichments indicates that oil-degrading bacteria were present in very low numbers and required a longer incubation time to bring about the changes observed in the chemical composition of this oil. It would also appear that "washing" (i.e. rinsing) the seaweed has a stimulatory effect on the oil-degrading capability of the microbial flora present. Not enough samples of seaweed were examined to see if there was any relationship between the variety of seaweed, or its geographic location or age and the degradation of oil. Similarly, not enough cobbles were examined to determine whether there was any correlation between geographic location, type of cobble or its position relative to tide and the degradation of oil. The surface area examined, at least between 10 cm^2 and 40 cm^2 for seaweed and between 35 cm^2 and 106 cm^2 for cobbles, does not appear to be correlated to the ability to utilize Prudhoe Bay oil.

While the generic composition of the original marine population (Tables 35, 38, 41) can range from low levels of many different genera to ones with only one or two genera predominating, the composition of oil-degrading populations consists of only one or two prominent generic types. Most oil-degrading populations of this type consist of members of the *Flavobacterium* genus with occasional populations showing high levels of members of the *Pseudomonas*, *Acinetobacter* or *Alcaligenes* genera. The absence of vibrios (common marine bacteria) as a significant proportion of oil-degrading cultures is due to their inability to grow on oil, as the medium used in this study readily supported the growth of members of the genus *Vibrio* (Table 43).

The concensus in the literature is that crude oil degradation in marine and terrestrial environments results primarily from bacterial activity. The literature on the role of fungi and yeasts in oil-degradation has been recently reviewed (4, 6, 36) and it was concluded that they could play a role in the removal of oil spilled in the environment. Fungi and yeasts (237 cultures) isolated by direct plating and enrichment techniques from northern Puget Sound and the Pt. Angeles area were screened by a stationary culture technique (11) for their ability to grow (i.e. utilize n-alkanes and isoprenoids) on Prudhoe Bay oil at 8°C or 20°C. The majority of these isolates, 76% at 20°C and 83% at 8°C, would not grow on Prudhoe Bay oil under the experimental conditions used in this study. Those which did grow were primarily members of the *Aspergillus*, *Penicillium* and *Verticillium* genera and in most cases they completely utilized the n-alkanes. Only three yeasts of the 74 isolated were capable of utilizing the n-alkanes present in Prudhoe Bay oil. None of the fungal or yeast cultures isolated were able to utilize the isoprenoids present in this oil. Twenty cultures identified as *Cladosporium resinae*, which has been associated with oiled marine environments (37), were isolated in this study. However, as previously reported (11) for terrestrial isolates and cultural collection stains, none of the marine *C. resinae* isolates were able to grow on Prudhoe Bay oil. Not enough fungi were obtained by the enrichment technique to compare its efficiency in isolating oil-degrading fungi and yeasts with the direct plating technique. Considering the selective pressures, e.g. low pH and low aeration required to enhance the recovery of fungi rather than bacteria from environmental samples, it is doubtful whether oil-degrading fungi and yeasts are competitive with bacteria in removing oil from aquatic or terrestrial environment under non-stressed environmental conditions. However there is a relatively high percentage of the fungal isolates (apparently restricted to a few genera) with the ability to utilize n-alkanes such that under conditions where bacterial growth is restricted these organisms could initiate the degradation of oil.

Studies on the rate of mineralization of Prudhoe Bay oil under non-nutrient-limiting conditions, using a gravimetric procedure (Figures 8, 10), showed maximum rates of 50.5, 13.5, 37.7 and 14.6 mg of oil removed per litre of water per day at 8°C for water column samples from E. Fidalgo, Ediz Hook (Pilot Station), Peabody Creek, and Dungeness Spit (No. 2) respectively. No degradation of this oil was observed under the conditions of this experiment with the water column sample taken from Pt. Partridge (April, 1979). Samples from E. Fidalgo (near Anacortes oil refineries) and Peabody Creek (southside of Pt. Angeles harbor), showed the highest rates of mineralization of oil and the shortest lag times (i.e. period before rapid oil utilization takes place). This is not unexpected as both these sites are probably being exposed to chronic low levels of hydrocarbon contamination. Sediments from March Pt. (near the Anacortes refineries) have been found to contain relatively high levels of saturate and aromatic hydrocarbons (40); summary in Appendix. This would result in a selective pressure being brought to play on the "normal" marine microbial flora and result in natural enrichment of hydrocarbon-degrading bacteria. As reported in the literature (13, 14), loss of weight of oil was brought about by the utilization of components of the saturate and aromatic fractions (Figures 9, 11). Little change is noted in the proportion of asphaltenes whereas the recovery of polar (i.e. N,S,O's) compounds increases with time. This latter observation suggests a conversion of non-polar hydrocarbons found in the saturate and aromatic fractions to polar compounds, probably via partial oxidation or co-oxidation (31). The data (Figures 9, 11) on the rates of change of these fractions suggest a gradual utilization of the aromatic compounds (e.g. Ediz Hook and E. Fidalgo water) whereas a definite lag phase followed by a period of very rapid utilization occurs with compounds present in the saturate fraction (e.g. n-alkanes) suggesting that there is an inherent ability present in the microbial flora in some water samples to utilize components present in the aromatic fraction. However an induction period is required before the rapid utilization of components in the saturate fraction occurs. The extensive utilization of Prudhoe Bay oil which occurs with prolonged incubation - that is, longer than the 28 days used in the activity survey (Figure 11) - indicates that the survey data has to be interpreted carefully. The results observed, therefore, are related to the length of the incubation period and/or the sample size used as source of inoculum. Increasing either or both variables would with some samples increase the degree of chemical change observed in recovered oil.

Oil-utilization is a biochemical characteristic which is widespread, although at markedly different levels, in the microbial flora found in this marine environment. This is reflected in the varied response, even with nutrient supplementation, of the microbial flora in different samples to bring about changes in the chemical composition of Prudhoe Bay oil.

Greater variation in gravimetric recovery studies was observed with samples from pristine areas, e.g. Ediz Hook (Pilot Station) and Dungeness Spit No. 2 than from areas likely to be subject to hydrocarbon contamination. This is probably a result of a heterogeneous distribution of microorganisms in the sample size used in pristine areas and could be overcome by using a larger sample size. Difficulties were also experienced with emulsion formation on long term incubation of oil-water mixtures which interfered with the recovery of the oil. Attempts to carry out gravimetric studies on sediment samples also were unsuccessful because of the formation of stable emulsions making quantitative recovery of oil impossible.

Investigations on the rate of mineralization using Prudhoe Bay oil "spiked" with one of n-[1-^{14}C]-hexadecane, [1-^{14}C]-naphthalene, [9-^{14}C]-phenanthrene or [9-^{14}C]-anthracene produced data on the fate of individual hydrocarbons in oil, whereas the gravimetric procedure determined total changes in the components of oil. The radiometric technique relies on the release of $^{14}CO_2$ from compounds specifically labelled with carbon-14. Thus the position of the label will be a factor in the time required and the amount of metabolism required before $^{14}CO_2$ is released. The dilution of added ^{14}C-hydrocarbons by the corresponding component in oil also has to be considered in the determination of the rates of removal of individual hydrocarbons. The contents of these hydrocarbons in Prudhoe Bay oil used for calculating rates of removal of individual hydrocarbons are taken from the current literature available to us.

The metabolism of n-alkanes can proceed via α, ω or internal oxidation (32). The first step in the α and ω terminal oxidation processes is the formation of a primary alcohol which is subsequently oxidized to an acid. Further pathways of catabolism of such acids, although not firmly established, probably proceed via β-oxidation to yield acetate units whose metabolism would yield $^{14}CO_2$. Regardless of the site of initial oxidation, many enzymatic steps, and thus time, are required to yield $^{14}CO_2$ from n-[1-^{14}C]-hexadecane. Pathways (33) involved in the catabolism of the aromatic compounds naphthalene, anthracene and phenanthrene by pseudomonads are summarized in Figure 23 (pseudomonads and related organisms predominate in oil-degrading enrichments). Many enzymatic steps are required before $^{14}CO_2$ will be released from the catabolism of these aromatic compounds. All of them, and naphthalene in particular, are labelled in symmetrical sites. Therefore the number of steps involved before the release of $^{14}CO_2$, and thus the time elapsed, will also depend on the site where the initial oxidation takes place relative to the ^{14}C-labelled carbon atom. But in all cases, cleavage and extensive catabolism of the aromatic ring(s) are required before $^{14}CO_2$ will be released. The shape of the $^{14}CO_2$ release curves for a given compound reflects the time required (i.e. lag) before the microbial flora adapts to the respective substrates and maximum rates of $^{14}CO_2$ evolution are observed. As these compounds are added in oil it is also possible that some differences observed in lags, rates of $^{14}CO_2$ released and recovery are a result of the response of the microbial flora to other compounds present in oil.

The rapid release of $^{14}CO_2$ from ^{14}C-labelled naphthalene by the microbial flora present in all water column samples tested (Figures 12, 13) indicates that such populations quickly adapt to the addition of this substrate. It is well established that, in the control and regulation of procaryotic (i.e. bacterial) metabolism, the substrate which supports the fastest growth rate will be used first and its use will inhibit the

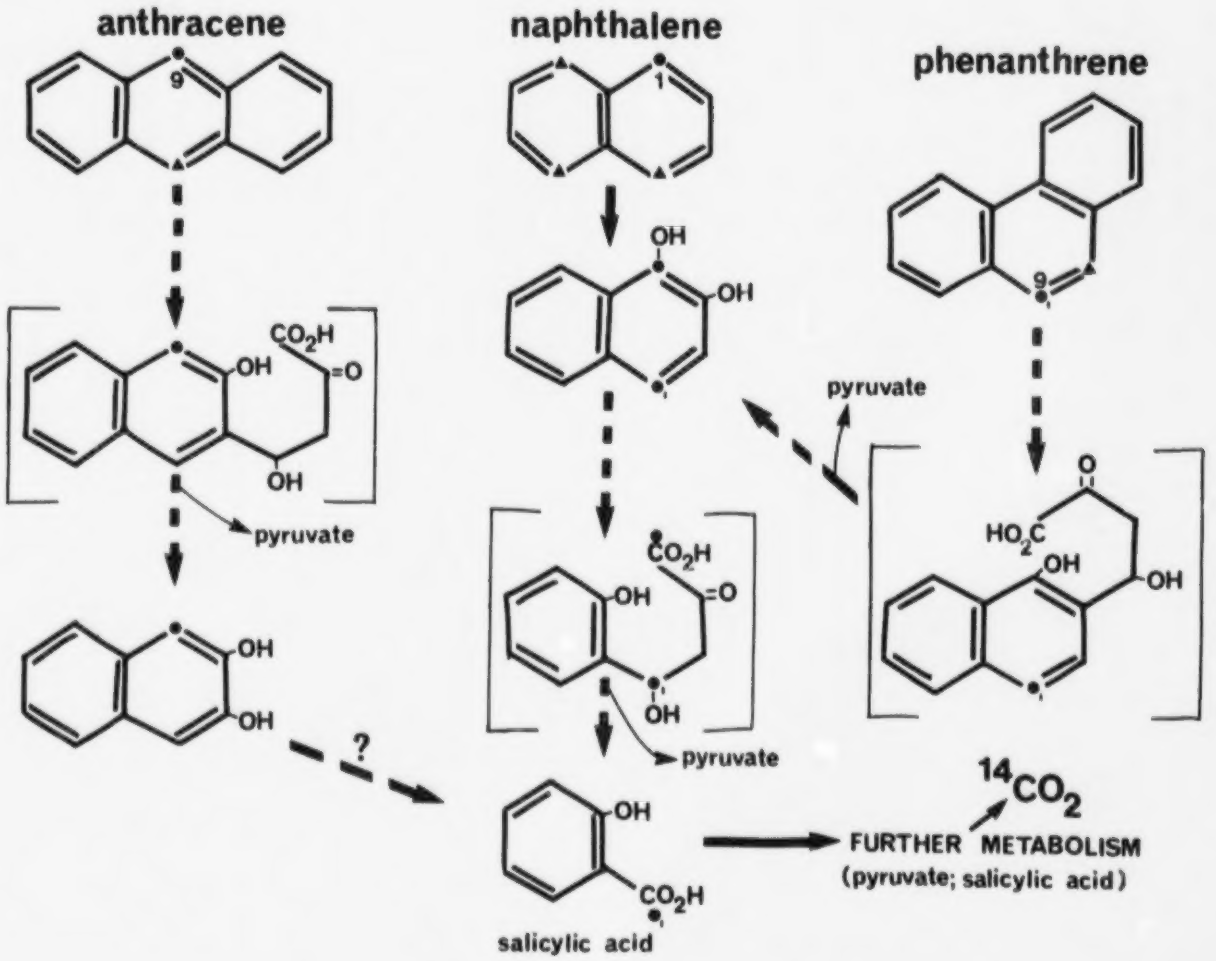
anthracene
9
pyruvate
OH
CO_2H
=O
?
naphthalene
1
CO_2H
pyruvate
salicylic acid
phenanthrene
9
HO_2C
O
FURTHER METABOLISM
(pyruvate; salicylic acid)
$^{14}CO_2$

Figure 23. Pathways of catabolism of anthracene, naphthalene and phenanthrene by pseudomonads (34): (C^{14} used in pathway; asymmetric sites, number of reactions between intermediates).

synthesis of the enzymes required to metabolize other more slowly utilized substrates. The microbial flora present in waters from areas likely to have been exposed, or being exposed, to chronic low level hydrocarbon contamination, i.e. E. Fidalgo and Peabody Creek, much more quickly adapt (as determined by length of incubation i.e. days before $^{14}CO_2$ is rapidly released) to the catabolism of hexadecane, anthracene and phenanthrene. In contrast, longer incubation times are required before the microbial flora present in other waters release $^{14}CO_2$ from these compounds. The differences in yields of $^{14}CO_2$ relative to the amount of ^{14}C-labelled substrate added reflects in part the differences in the catabolic pathways used and/or the degree of assimilation (i.e. incorporation of carbon into cell material) which occurs with the different substrates and populations. For example the high degree of recovery of carbon-14 from [9-^{14}C]-phenanthrene as $^{14}CO_2$ indicates that this carbon is readily released as $^{14}CO_2$ whereas the lower recoveries from [1-^{14}C]-hexadecane could reflect a higher degree of assimilation taking place. In addition, since there is an increase in the polar components of crude oil as a result of microbial catabolism of oil, some of the ^{14}C-labelled substrates could be converted by partial oxidation or co-oxidation to such compounds. Therefore the total ability of the microbial flora present to remove specific hydrocarbons in oil will not be known until the distribution of carbon-14 in other potential products of metabolism is investigated.

The $^{14}CO_2$ release patterns from the microbial flora present in the sub-tidal sediments (Figure 14) show a rapid release of $^{14}CO_2$ from [1-^{14}C]-naphthalene. In contrast to the $^{14}CO_2$ release patterns from the water column samples taken at the same time, where Peabody Creek was the most active, these results show that the sub-tidal sediment from Ediz HooK was more active on anthracene and hexadecane. Very little difference exists however between the $^{14}CO_2$ release patterns brought about by Peabody Creek and Ediz Hook on phenanthrene.

The highest rates (not related to lag) of removal of these compounds from Prudhoe Bay oil by both water column and sub-tidal sediments were observed for phenanthrene (Tables 16, 17, 18). This was followed by naphthalene with hexadecane generally being removed at a slightly higher rate than anthracene. The length of lag observed (i.e. time before a rapid rate of $^{14}CO_2$ release is achieved) (Tables 16, 17, 18) confirms the observation from Figures 12, 13, 14 that naphthalene is the most readily utilizable hydrocarbon tested. The lengths of lags observed with the other substrates varies to some degree with the type of sample being studied (i.e. water column versus sub-tidal sediment). The ability of sub-tidal flora to catabolize polycyclic aromatic hydrocarbons, even under highly aerobic conditions, is important as it was recently shown (34) that ^{14}C-anthracene added to two 80-litre pond microcosms rapidly accumulated in the sediment. Therefore the presence of a sub-tidal microbial flora capable of catabolizing such compounds is very important if such pollutants are going to be removed or maintained at low levels in this area of the marine environment. The release of $^{14}CO_2$ from "spiked" oils by microbes found in beach samples (Table 19) indicates that they are capable of catabolizing hexadecane and naphthalene (samples were not tested on [9-^{14}C]-anthracene or [9-^{14}C]-phenanthrene).

Oil-degrading activity of microbial flora in this study is based on changes occurring in the n-alkane and isoprenoid components of the saturate fraction of oils. It has been shown however (35, 15) that aromatic components, in particular mon-, di- and tri-cyclic ring systems, are readily removed from oil by microbial action. This was confirmed by some of the gravimetric data obtained and in the studies using carbon-14 "spiked" Prudhoe Bay oil. However the gravimetric study reflects gross changes in oil as a result of microbial catabolism and the radiometric technique provides information on the fate of an individual hydrocarbon (albeit in the presence of other components of crude oil). Therefore an investigation of the utilization patterns of saturates and aromatics of Prudhoe Bay oil was undertaken using glass capillary gas chromatography which requires extensive pre-fractionation of oil but provides detailed information on other changes taking place in these fractions of Prudhoe Bay oil.

The rates of utilization of saturate and aromatic components as a function of time at 8°C in the presence of exogenous nutrients was investigated using water column samples from E. Fidalgo. No changes were noted in either the n-alkane or aromatic fractions of recovered oil (data not reported) after 3 days incubation. Very few changes were detected in the saturate fraction (data not reported) after 6 days incubation whereas the more volatile components like substituted benzenes, 1-methyl- and 2-methylnaphthalenes were being utilized at this time (Figures 16, 17). However, between day 6 and 10 the n-alkanes and isoprenoids were completely used whereas only the substituted benzenes and methylnaphthalenes had been used in the aromatic fraction and the degradation of dimethylnaphthalenes, dibenzothiophene and phenanthrene had been initiated. Extensive utilization of these aromatic components had occurred after 14 days incubation although the phenanthrene and anthracene peaks were still discernible. After 27 days incubation in the presence of exogenous nutrients the glass capillary G.C. profile (Figure 18) shows that extensive metabolism of aromatic components has taken place as a result of microbial activity. A comparison of the G.C. profiles of uninoculated controls (Figures 17 and 18) shows that extensive loss of these components also occurs via volatilization. These results confirm the previous data which indicated aromatic catabolism proceeds prior to the extensive, spectacular catabolism of n-alkanes and isoprenoids, and continues after the saturate components have been removed from the oil.

The effect of the presence or absence of exogenous nutrients on the changes in the chemical composition of the saturate and aromatic components of Prudhoe Bay oil was studied using water column samples from Peabody Creek (Pt. Angeles harbor), E. Fidalgo (near Anacortes refineries), and Pt. Partridge. The glass capillary profiles of these fractions (Figure 27) show that in the absence of added nitrogen and phosphorus the microbial flora present in water column samples from Peabody Creek and Pt. Partridge brought about little change in the n-alkane and isoprenoid profile (Figure 21). In contrast, water from E. Fidalgo brought about quite readily detectable changes in the n-alkanes present in Prudhoe Bay oil. The profiles of the corresponding aromatic fraction (Figure 20) show more changes in the aromatic fraction of the oil recovered from the Peabody Creek and Pt. Partridge water samples than from E. Fidalgo. In the presence of exogenous nutrients the microbial flora present in water samples from Peabody Creek and E. Fidalgo completely utilized the n-alkanes and isoprenoids whereas water from Pt. Partridge only partially removed the n-alkanes and did not utilize the isoprenoids present in Prudhoe Bay oil (Figure 21). Similarly, greater changes were brought about in the aromatic components by the microbial flora present in water from Peabody Creek and E. Fidalgo than by water from Pt. Partridge (Figure 19) where the anthracene peak is still discernible.

These results indicate that catabolism of saturates and aromatics do take place in the absence of added nutrients although at a very much slower rate. The degree of utilization of these fractions also varies with source of the sample. Water from E. Fidalgo, which is adjacent to oil refineries, brought about changes in the saturate fraction but was less active on the aromatic components. In contrast, water from the pristine Pt. Partridge area and Peabody Creek (a heavily used commercial, industrial area) brought about greater changes in the aromatic components than in the saturate fraction.

The chemical composition of oil has been shown to affect its biodegradability (16, 22). Since refineries in the Puget Sound area, particularly those near Anacortes, receive oil from areas of the world other than the north slope of Alaska an investigation of the biodegradability of representative oils was undertaken. The data (Table 21) indicate that the n-alkanes in Murban and Seria oils were readily catabolized by stock, laboratory oil-degrading cultures from the Puget Sound area. These populations were not able to bring about similar changes in Minas oil which is a solid at 8°C. The n-alkanes in Seria oil were readily catabolized by the microbial flora present in waters and beach samples from the northern Puget Sound and Port Angeles area. However Murban oil was not as readily metabolized and Minas oil was only utilized by the microbial flora present in beach samples from Peabody Creek (Pt. Angeles harbor) and Dungeness Spit No. 2. These results indicate that attention must be paid to the type of oil being shipped, since biodegradability differs considerably. A spill of Minas oil, even though it is not readily degraded, would not be a problem as at temperatures prevalent in the air and waters of this area it would be a solid and thus physically removable. However it has been noticed that under the laboratory conditions used this "oil" occasionally formed "tar-balls" which sank in the artificial seawater solution used in these experiments.

During the course of the sub-tidal oil-degrading experiment conducted by the Battelle laboratories at Sequim Bay the incidence of oil-degrading heterotrophs increased in oil treated materials until they become a significant proportion (up to 10%) of the heterotrophic population (Table A8). However the fact that a similar shift, at a slightly lower level, occurred in the non-oiled plots suggests that oil cross-contamination of plots occurred.

The incidence of oil-degrading heterotrophs initially was low and similar values were found for the fine and coarse material examined in the preliminary study. This incidence changed quite rapidly in the coarse material, whereas a considerably longer period of time was required to bring about a similar change in the fine material. This is a very sound approach to studying the effect of oil on sub-tidal biological activities. However from a microbial aspect more samples are required to follow the changing trends in the activities of the oil-degrading flora present as well as the use of more sensitive techniques. For example the incorporation of carbon-14 labelled hydrocarbons in the oil-degrading enumeration procedure (10) and the use of the radiometric technique employed in this investigation would be useful. These sensitive procedures coupled with G.C. glass capillarly analysis of the aromatic and saturate fractions of the oil recovered periodically should provide the data required to interpret changes in the microbial flora.

The oil-degrading capability of the microbial flora in the Duwamish River flowing through south Seattle and entering Puget Sound at Elliott Bay was investigated. The numbers of bacterial, yeast and fungal colony forming units (Table 44) are similar to those found in other areas of these coastal waters studied. All samples (both water column and sediments) readily utilized the n-alkanes and isoprenoids present in Prudhoe Bay oil (Table 45) under these experimental conditions. Significant changes also were noted in enrichments which did not receive a nitrogen and phosphorus supplement. There is a relatively high level of oil-degrading activity amongst the microbial flora present (even though total viable numbers are similar) in this water system as compared to that in other sites investigated. This undoubtedly is a result of the presence of relatively high levels of hydrocarbons and greases in this environment (hydrocarbon sheens were readily visible on the surface of the water at many of the sites sampled and hydrocarbon sheens were visible in the sediment samples taken from this area). Their presence would result in a very active oil-degrading component being present and functional in the micro-flora of this water system.

The information presented in this and the previous report (8) indicates a viable but ubiquitous distribution of oil-degrading microorganisms in the water, beaches, inter-tidal and sub-tidal sediments of northern Puget Sound and the southern shores of the Strait of Juan de Fuca. Samples from areas which have been or are subject to chronic, low-level contamination by hydrocarbons(e.g. oil refineries or commerical traffic) have a higher rate of activity than those from other areas. As noted in other studies the oil-degrading activity of these microorganisms is limited by the nitrogen and/or phosphorus content of the various marine environments found in these areas. It is doubtful whether "fertilizing" marine oil spills even with oleophilic fertilizers containing nitrogen and phosphorus (37) can be effectively and efficiently used on open water spills. However such fertilizers could be of use in treating oil spills reaching inter-tidal and beach areas. The ability of the marine environment in this area to recover from an oil spill is illustrated by the fate of an oil, classed as a No. 5 fuel oil, in the Alert Bay area of northern Vancouver Island (38, 39). Studies (39) over a 4-year period followed the fate of the oil in a bay area (Reserved Bay, Pearse Island group) which was not subjected to clean up procedures. The n-alkanes were removed during the first year following the spill whereas pristane and phytane were not completely utilized until the fourth year. The distribution pattern of residual hydrocarbons (tentatively identified as oil pentacyclic terpenes) recovered in the fourth year had a distribution pattern similar to that of the original oil. The inter-tidal biota which was initially adversely affected by the spill was well on its road to recovery within a year of the spill. These observations suggest that normal nutrient cycling of nitrogen in particular, and the level of phosphorus, are sufficient to support the oil-degrading activities of the indigenous microbial population and that over an extended period of time significant microbial degradation of oil will take place. This action together with the normal physical-chemical processes (e.g. photolysis, evaporation, dissolution, etc.) involved in removing oil in the marine environment were able to remove most of the oil spilled within a 4-year period.

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APPENDIX

Composition of Microbiological Media

(i) Artificial Seawater Solution

NaCl	23.40 g
KCl	0.75 g
$MgSO_4 \cdot 7H_2O$	7.00 gm
Distilled water	1000 ml
pH	7.3

(ii) Basal Marine Agar (for viable cell counts; storage of bacterial cultures and for oxidase and catalase tests).

proteose peptone #3 (Difco)	1 g
yeast extract (Difco)	1 g
agar (Difco purified)	20 g
artificial seawater	1000 mL
pH adjusted to 7.5 before autoclaving	
final pH	7.3

(iii) O.F. Medium for Sugar Utilization Tests

Solution A -	NaCl	23.40 g
	KCl	0.75 g
	$MgSO_4 \cdot 7H_2O$	7.00 g
	yeast extract (Difco)	1.00 g
	Bromthymol blue	0.03 g
	agar (Difco purified)	15.0 g
	distilled water	750 mL
Solution B -	glucose or lactose	10.0 g
	distilled water	200 mL
Solution C -	$NH_4H_2PO_4$	0.50 g
	K_2HPO_4	0.50 g
	distilled water	50 mL

The pH of all solutions was adjusted to 7.15 before autoclaving. After cooling to 50°C solutions A and B were mixed, solution C added and mixed and then the medium was dispensed into Petri plates.

(iv) Saline Sabouraud's agar - 65 g of Sabouraud dextrose agar (Difco) added to 1000 ml of artificial seawater solution; after minimum sterilization the medium was cooled to 50°C and 100 mg of streptomycin and 60,000 units of penicillin-G (both filter sterilized) added to 1 L of medium.

(v) Malt agar - prepared as per Difco's instructions.

(vi) TCBS agar - 89 g added per 1 L of artificial seawater solution.

(vii) Marine agar 2216 - 37.4 g of purified Difco agar added per 1 L of distilled water.

(viii) Amino acid medium for Most Probable Number determinations. Thirty g of vitamin-free cosamino acids added per 1 L of artificial seawater solution.

(ix) Modified Butlin's for sulfide-generating bacteria.

Solution A -	NH_4Cl	1.00	g
	Na_2SO_4	2.00	g
	$MgSO_4 \cdot 7H_2O$	0.02	g
	Sodium lactate	1.5	mL (60% syrup)
	yeast extract (Difco)	1.0	g
	K_2HPO_4	0.5	g
	artificial seawater	1000	mL

Ten mls dispensed per 18x150 mm test tube and 2 iron finishing nails added per tube for poising the medium before sterilization.

Solution B - 3 drops of freshly prepared 10% Na_2SO_3 solution (sterilized by filtration) added to each tube of medium A prior to inoculation.

(x) Nitrogen-and phosphorus-containing solution used for nutrient supplementation.

10% K_2HPO_4	420	mL
10% KH_2PO_4	180	mL
NH_4NO_3	60	g

pH adjusted to 7.3 with 10N NaOH prior to autoclaving; used at the rate of 1 ml/100 ml of natural or artificial seawater medium.

Biochemical Tests for Bacterial Identification

(a) Oxidase reagent - 1% solution of N,N,N',N'-tetramethyl-p-phenylenediamine-dihydrochloride; 1 to 3 drops added to colonies growing on basal marine agar.

(b) Catalase reagent - 1 to 3 drops of freshly prepared 3% H_2O_2 added to colonies growing on basal marine agar.

TABLE A1. DISSOLVED OXYGEN, SALINITY AND TEMPERATURES OF SAMPLES FROM NORTHERN PUGET SOUND.

Site	Date	Water Column		Temperature (°C)	
		Dissolved Oxygen (ppm)	Salinity (o/oo)	beach	water
Samish Island	Aug. 14/78	8.1	21.5	15	14
	Nov. 21/78	11.2	22.0	3	7
	April 2/79	9.8	21.8	13	10
E. Fidalgo	Aug. 14/78	3.6	22.6	15	15
	Nov. 21/78	11.8	22.5	8	5
	April 2/79	9.4	24.3	17	12
Pt. Partridge	Aug. 14/78	8.5	24.8	14	13
	Nov. 21/78	9.5	24.0	2	8
	April 2/79	7.8	24.9	25	12
	Aug. 21/79	9.1	28.3	-[a]	14

[a] sample not taken

TABLE A2. DISSOLVED OXYGEN, SALINITY AND TEMPERATURES OF SAMPLES FROM PT. ANGELES AREA.

Site	Date	Water Column				Temperature (°C)		
		Dissolved Oxygen (ppm)		Salinity (o/oo)				
		top	bottom	top	bottom	beach	water column	bottom
Freshwater Bay	Oct. 2/78	10.6	8.4[a]	19.0	20.5[a]	-[b]	12	12[a]
	Jan. 15/79	10.6	-	27.8	-	5	-	-
	June 19/79	9.1	-	21.9	-	14	-	-
Pt. Angeles-Ediz Hook #1	Oct. 2/79	9.9	7.9[c]	21.4	21.0[c]	-	14	14[c]
	Jan. 15/79	9.9	-	27.1	-	2	6	-
	June 19/79	9.3	-	21.0	-	19	11	-
Pt. Angeles-Ediz Hook #2	Oct. 2/78	7.8	-	26.0	-	13	14	-
	Jan. 15/79	12.6	-	27.6	-	6	6	-
	June 19/79	14.0	-	22.8	-	18	11	-
Pt. Angeles-Southside (Marina)	Oct. 2/78	8.5	-	20.1	-	13	16	-
	Jan. 15/79	11.2	-	27.4	-	-	6	-
	June 19/79	11.8	-	23.3	-	19	11	-
Pt. Angeles-Southside-(Peabody Creek)	Oct. 2/78	8.0	7.3[d]	24.6	21.3[d]	11	13	15[d]
	Jan. 15/79	11.1	-	16.2	-	4	5	-
	June 19/79	13.6	-	18.3	-	18	12	-

TABLE A2. continued ....

Site	Date	Water Column				Temperature (°C)		
		Dissolved Oxygen (ppm)		Salinity (o/oo)				
		top	bottom	top	bottom	beach	water column	bottom
Pt. Angeles-Southside (Red Lion Inn)	Oct. 2/78	7.8	-	26.1	-	12	13	-
	Jan. 15/79	12.2	-	27.3	-	5	6	-
	June 19/79	11.7	-	18.2	-	16	12	-
West of Morse Creek	Oct. 2/78	8.2	-	24.7	-	11	17	-
	Jan. 15/79	14.4	-	27.1	-	4	7	-
	June 20/79	12.6	-	20.5	-	-	15	-
West of Green Point	Oct. 2/78	11.2	-	25.0	-	11	17	-
Green Pt. #1	Oct. 2/78	8.3	-	24.9	-	11	16	-
Green Pt. #2	Oct. 2/78	9.1	-	25.1	-	14	16	-
Green Pt. #3	Oct. 2/78	8.8	-	24.3	-	21	17	-
	Jan. 16/79	10.8	-	27.0	-	6	7	-
	June 19/79	10.0	-	23.0	-	18	13	-

TABLE A2. continued ....

Site	Date	Water Column				Temperature (°C)		
		Dissolved Oxygen (ppm)		Salinity (o/oo)				
		top	bottom	top	bottom	beach	water column	bottom
Dungeness Spit #1	Oct. 2/78	8.4	-	24.9	-	-	17	-
	Jan. 16/79	11.2	-	26.9	-	5	6	-
	June 19/79	10.9	-	23.3	-	18	13	-
Dungeness Spit #2	Oct. 2/78	10.4	-	21.5	-	21	17	-
	Jan. 16/79	10.1	-	27.1	-	4	6	-
	June 19/79	9.9	-	23.0	-	19	14	-
Dungeness Lagoon	June 19/79	10.6	-	22.8	-	23	15	-

[a] bottom water - 7.62 meters depth

[b] no sample taken

[c] bottom water - 9.14 meters depth

[d] bottom water - 15.24 meters depth

TABLE A3. CHEMICAL ANALYSIS OF WATER SAMPLES FROM NORTHERN PUGET SOUND SITES.

Date	Site	Nitrogen (μg/L)		Total orthophosphate (μg/L)	Total organic carbon (mg/L)	Greases and oils (μg/L)	Suspended solids (mg/L)
		NO_3^-	NH_4^+				
August 13-14, 1978	Samish Island	70	750	80	15	-[a]	-
	E. Fidalgo	70	770	100	16	-	-
	Pt. Partridge	230	540	70	13	-	-
April 2-3, 1979	Samish Island	150	90	76	9	-	-
	E. Fidalgo	78	130	1740	13	-	-
	Pt. Partridge	120	-	46	2	-	-
June 18-20, 1979	Samish Island	-	-	-	-	170	13.4
	E. Fidalgo	-	-	-	-	500	13.8
	Pt. Partridge	-	-	-	-	260	19.3

[a] not analyzed

TABLE A4. CHEMICAL ANALYSIS OF BEACH SAMPLES FROM NORTHERN PUGET SOUND SITES.

Date	Site	Nitrogen (µg/g)		Total orthophosphate (µg/g)	Greases and solids (mg/g)
		NO_3^-	NH_4^+		
August 13-14, 1978	Samish Island	3.7	0.02	1.1	0.16
	E. Fidalgo	ND[a]	0.003	0.7	0.06
	Pt. Partridge	0.16	0.007	0.02	0.07
April 2-3, 1978	Samish Island	3.3	2.3	2.4	-[b]
	E. Fidalgo	3.0	1.3	360	-
	Pt. Partridge	2.5	1.2	0.11	-

[a] not detected by method used

[b] not analyzed

TABLE A5. CHEMICAL ANALYSIS OF WATER SAMPLES FROM PT. ANGELES AREA SITES.

Date	Site	Nitrogen (µg/L)		Total orthophosphate (µg/L)	Total organic carbon (mg/L)	Greases and oils (µg/L)	Suspended solids (mg/L)
		NO_3^-	NH_4^+				
October 1-3, 1978	Freshwater Bay	120	130	51	6.2	-[a]	-
	Pt. Angeles-Ediz Hook #1	170	ND[b]	34	5.3	-	-
	Pt. Angeles-Ediz Hook #2	230	350	31	4.5	-	-
	Pt. Angeles-Southside Marina	260	ND	37	3.6	-	-
	Pt. Angeles-Peabody Creek	270	100	40	3.6	-	-
	Pt. Angeles-Red Lion Inn	270	100	44	4.3	-	-
	West of Morse Creek	310	180	41	3.3	-	-
	West of Green Point	290	250	41	3.3	-	-
	Green Point #1	400	ND	44	5.2	-	-
	Green Point #2	410	240	50	3.7	-	-
	Green Point #3	490		46	4.2	-	-
	Dungeness Spit #1	380	110	43	3.5	-	-
	Dungeness Spit #2	400	220	44	3.7	-	-

TABLE A5. continued .....

Date	Site	Nitrogen (μg/L)		Total orthophosphate (μg/L)	Total orthophosphate (mg/L)	Greases and oils (μg/L)	Suspended solids (mg/L)
		NO_3^-	NH_4^+				
June 18-20, 1979	Pt. Angeles-Ediz Hook #1	110	260	51	4	260	8.2
	Pt. Angeles-Peabody Creek	ND	210	43	15	360	18.6
	Dungeness Spit #2	ND	580	65	7	300	115

[a] not analyzed

[b] not detected by method used

TABLE A6. CHEMICAL ANALYSIS OF BEACH AND SUB-TIDAL SAMPLES FROM PT. ANGELES AREA SITES.

Date	Sample	Site	Nitrogen (μg/g)		Total orthophosphate (μg/g)	Greases and oils (mg/g)
			NO_3^-	NH_4^+		
October 1-3, 1978	Beach	Freshwater Bay	0.29	9.0	9.9	0.034
		Pt. Angeles-Ediz Hook #1	1.5	2.9	0.93	0.094
		Pt. Angeles-Ediz Hook #2	0.85	4.6	1.2	0.160
		Pt. Angeles-Southside Marina	0.7	5.9	5.1	0.052
		Pt. Angeles-Peabody Creek	0.7	4.1	9.8	0.091
		Pt. Angeles-Red Lion Inn	0.45	3.5	12	0.046
		West of Morse Creek	1.8	5.2	ND[a]	0.009
		West of Green Point	2.7	7.7	0.07	0.010
		Green Point #1	0.2	4.0	ND	0.010
		Green Point #2	0.03	4.2	0.10	0.110
		Green Point #3	1.1	14	4.4	0.007
		Dungeness Spit #1	0.1	ND	1.1	0.005
		Dungeness Spit #2	1.0	12	0.36	0.025

TABLE A6. continued....

Date	Sample	Site	Nitrogen (μg/g)		Total orthophosphate (μg/g)	Greases and oils (mg/g)
			NO_3^-	NH_4^+		
October 1-3, 1978	Sub-tidal	Freshwater Bay	0.29	14	34	0.16
		Pt. Angeles-Ediz Hook #1	0.038	34	21	0.59
		Pt. Angeles-Peabody Creek	0.13	13	47	0.37
June 18-20, 1979	Beach	Pt. Angeles-Ediz Hook #1	1.9	ND	3.5	0.06
		Pt. Angeles-Peabody Creek	2.5	4.8	5.0	0.33
		Dungeness Spit #1	1.4	ND	0.79	0.01
	Sub-tidal	Pt. Angeles-Ediz Hook #1	6.3	13	150	0.42
		Pt. Angeles-Peabody Creek	8.4	ND	250	0.50
		Dungeness Spit #1	2.9	ND	5.0	0.01

[a] not detected by methods used

TABLE A7. MOST PROBABLE NUMBERS (±1 STANDARD DEVIATION) OF HETEROTROPHS, OIL-DEGRADING HETEROTROPHS AND AUTOTROPHS - BATTELLE EXPERIMENTS (SEQUIM BAY - LAGOON II, 1978-1979)

			Most Probable Numbers[a]				
Particle Size	Date	Sample	Heterotrophs[b]	Average ±1 S.D.[c]	Oil-Degrading Heterotrophs[b]	Average ±1 S.D.[c]	Autotrophs
Fine[d]	Summer 1978- (preliminary)	Treated	$0.5\text{-}5.6\times10^4$ $1.5\text{-}16.2\times10^5$ $1.0\text{-}10.9\times10^5$	$2.79(\pm2.4)\times10^5$	$0.7\text{-}7.6\times10^1$ $0.7\text{-}7.6\times10^2$ $2.4\text{-}26.8\times10^1$	$1.10(\pm1.1)\times10^2$	$<10^1$[e]
		Untreated	$1.5\text{-}16.2\times10^6$ $0.4\text{-}4.3\times10^5$ $0.3\text{-}3.6\times10^7$	$5.34(\pm5.4)\times10^6$	$0.7\text{-}7.6\times10^1$ $0.2\text{-}2.6\times10^1$	$1.54(\pm1.1)\times10^1$	$<10^1$
	Summer 1978	Treated	$1.5\text{-}16.2\times10^5$ $0.7\text{-}7.6\times10^6$ $0.7\text{-}7.9\times10^8$	$8.09(\pm13.7)\times10^7$	$0.7\text{-}7.3\times10^3$ $0.5\text{-}5.6\times10^4$ $1.5\text{-}16.2\times10^4$	$2.27(\pm2.4)\times10^4$	$<10^1$
		Untreated	$1.5\text{-}16.2\times10^6$ $2.4\text{-}26.1\times10^6$ $0.4\text{-}4.3\times10^6$	$4.70(\pm3.3)\times10^6$	$0.7\text{-}7.6\times10^3$ $0.5\text{-}5.6\times10^4$ $2.0\text{-}23.1\times10^2$	$6.67(\pm9.0)\times10^3$	$<10^1$
	Summer 1979	Treated	$2.4\text{-}26.1\times10^3$ $0.3\text{-}3.6\times10^4$ $0.3\text{-}3.6\times10^4$ $0.5\text{-}5.6\times10^4$ $2.4\text{-}26.1\times10^3$ $1.5\text{-}16.2\times10^3$	$7.83(\pm5.5)\times10^3$	$2.1\text{-}23.1\times10^1$ $2.1\text{-}23.1\times10^3$ $1.5\text{-}16.2\times10^2$ $0.4\text{-}4.3\times10^3$ $1.5\text{-}16.2\times10^2$ $1.0\text{-}10.9\times10^2$	$3.99(\pm6.4)\times10^2$	$<10^1$

TABLE A7 continued ....

Particle Size	Date	Sample	Most Probable Numbers[a]				
			Heterotrophs[b]	Average ±1 S.D.[c]	Oil-Degrading Heterotrophs[b]	Average ±1 S.D.[c]	Autotrophs
Fine[d]	Summer 1979	Untreated	0.7-7.3x10^{4} 0.4-4.3x10^{4} 1.5-16.2x10^{3} 1.5-16.2x10^{3} 0.7-7.6x10^{4} 1.4-15.2x10^{3}	8.65(±7.2)x10^{3}	1.0x10.9x10^{2} 0.7-6.9x10^{2} 1.4-15.2x10^{1} 1.4-15.2x10^{1} 0.7-7.6x10^{2} 0.5-5.6x10^{1}	7.06(±5.1)x10^{2}	<10^{1}
Coarse[f]	Summer 1978 (preliminary)	Treated	0.7-7.6x10^{6} 2.5-27.7x10^{7} 0.4-4.3x10^{6}	2.92(±4.7)x10^{7}	0.7-7.6x10^{1} 0.7-7.6x10^{1} 1.0-10.9x10^{1}	1.94(±1.6)x10^{2}	<10^{1}
		Untreated	1.5-16.2x10^{7} 0.4-4.3x10^{6} 0.4-4.3x10^{8}	6.01(±6.5)x10^{7}	0.4-4.3x10^{2} 0.7-7.6x10^{1} 0.4-4.3x10^{2}	9.4(±6.1)x10^{1}	<10^{1}
	Summer 1978	Treated	2.4-26.1x10^{5} 1.4-15.2x10^{5} 0.4-4.3x10^{5}	4.60(±3.3)x10^{5}	0.4-4.6x10^{5} 1.5-16.2x10^{2} 2.4-26.1x10^{2}	4.71(±8.0)x10^{4}	<10^{1}
		Untreated	2.4-26.1x10^{5} 0.4-4.3x10^{6}	1.05(±0.4)x10^{6}	0.7-7.6x10^{4} 2.4-26.1x10^{1}	1.19(±1.6)x10^{4}	<10^{1}

TABLE A 7 continued ....

Particle Size	Date	Sample	Most Probable Numbers[a] Heterotrophs[b]	Average ±1 S.D.[c]	Oil-Degrading Heterotrophs[b]	Average ±1 S.D.[c]	Autotrophs
Coarse[f]	Summer 1979	Treated	0.4-4.3x10^{3} 1.5-16.2x10^{3} 0.5-5.6x10^{4} 1.5-16.2x10^{3} 0.3-3.6x10^{4} 2.4-26.1x10^{3}	9.95(±4.1)x10^{3}	0.7-7.6x10^{2} 0.5-5.6x10^{3} [g] 0.7-7.6x10^{2} 0.4-4.3x10^{2} 0.7-7.3x10^{1} 2.4-26.1x10^{1}	5.36(±4.6)x10^{2}	<10^{1}
		Untreated	0.7-7.6x10^{4} 2.4-26.1x10^{3} 1.0-10.9x10^{3} 1.5-16.2x10^{3} 2.4-26.1x10^{3} 1.5-16.2x10^{3}	1.21(±0.86)x10^{4}	2.1-23.1x10^{3} 2.1-23.1x10^{4} 1.5-16.2x10^{4} 0.4-4.3x10^{4} 1.5-16.2x10^{3} 1.0-10.9x10^{7}	1.47(±1.3)x10^{2}	<10^{1}

[a] 28 days incubation at 20-22°C

[b] 95% confidence limits

[c] average of individual MPN's ±1 standard deviation

[d] fine particle size material from Protection Island

[e] lower detection limits

[f] coarse particle size material from Sequim Bay

[g] not used in average (±1 S.D.) calculation

TABLE A8. RATIOS OF OIL-DEGRADING HETEROTROPHS/HETEROTROPHS FOR BATTELLE EXPERIMENT (SEQUIM BAY-LAGOON II, 1978-1979).

Particle Size	Sample		Average MPN's		%
			Heterotrophs	Oil-Degrading Heterotrophs	Oil-Degrading Heterotrophs
Fine	Preliminary	Treated	$3\text{x}10^5$	$1\text{x}10^2$	0.033
		Untreated	$5\text{x}10^6$	$2\text{x}10^1$	0.0004
	Summer- 1978	Treated	$8\text{x}10^7$	$2\text{x}10^4$	0.025
		Untreated	$5\text{x}10^6$	$7\text{x}10^3$	0.14
	Summer- 1979	Treated	$8\text{x}10^3$	$4\text{x}10^2$	5.0
		Untreated	$9\text{x}10^3$	$7\text{x}10^2$	7.8
Coarse	Preliminary	Treated	$3\text{x}10^7$	$2\text{x}10^2$	0.0007
		Untreated	$6\text{x}10^7$	$9\text{x}10^1$	0.0002
	Summer - 1978	Treated	$5\text{x}10^5$	$5\text{x}10^4$	10.0
		Untreated	$1\text{x}10^6$	$1\text{x}10^4$	1.0
	Summer - 1979	Treated	$1\text{x}10^4$	$5\text{x}10^2$	5.0
		Untreated	$1\text{x}10^4$	$1\text{x}10^2$	1.0

TABLE A9. SUMMARY OF HYDROCARBON ANALYSES (40) OF SELECTED NORTHERN PUGET SOUND AND PT. ANGELES HARBOR AREA SEDIMENTS.

	ng/g of sediment			
	saturates (aromatics)			
Site	Soring	Summer	Fall	Winter
Dungeness	24[a]	83	16	56
Spit	(2.6)[a]	(4.6)	(0)	(0)
Dungeness	(860)[b]	(340)[a]	620	787
Three Crabs	(61)[b]	(26)[a]	(0)	(32)
Ediz	390[a]	301	228	262
Hook	(33)[a]	(17)	(0)	(21)
Whidby	7.7[a]	128	23	27
Island	(4.2)[a]	(6.2)	(2.0)	9
March	480[c]	280[a]	282	207
Point	(160)[c]	(220)[a]	(60)	(84)

[a] average of two values

[b] average of 3 values

[c] average of 4 values